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THE
ANATOMICAL RECORD

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A CRITIQUE OF THE CYTOLOGICAL METHOD: DETERMINING THE STRUCTURE OF LIVING CELLS FROM FIXED ONES

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INTRODUCTION

1. The danger of misinterpreting fixed cells due to coagulation artifacts

> At this day we no longer need the admonition that we must maintain a critical attitude towards all conceptions of protoplasm based on the study of fixed and stained sections—that is to say, on the study of cells that have been subjected to the barbarous insults of coagulation, dehydration, cooking in hot paraffin, embedding, sectioning, staining, and mounting in Canada balsam. Our faith in the results obtained by the practice of this kind of black magic is now less confiding than it was in a younger and more credulous age. If we do not tread warily amid the pitfalls of cytological technique it is not for lack of warning, though I must admit that even now that warning is not always sufficiently heeded.

This statement of Prof. E. B. Wilson ('26) but reemphasizes an attitude that has been expressed from time to time for over half a century. Between 1875 and 1900, such men as Flemming, Berthold, Schwarz, Fischer, Bütschli, and Hardy left nothing undone or unsaid to point out the dangers of ascertaining the structure of living cells from fixed ones due to coagulation artifacts.

In a broad sense, every coagulation product is an artifact of the living structure, since the one is dead while the other is alive, and since the chemical composition of the coagulated component differs from the living one, owing to its union with the reagent. In cytological studies, however, the word

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artifact is used in a narrower sense, referring to those fixation images that give misleading and distorted pictures of the morphology of living components and of their relations with other structures. Cells containing artifacts are frequently spoken of as 'abnormal' or 'poorly fixed,' in contrast to those that are 'normal' or 'well fixed,' i.e., those that yield accurate information concerning the morphology of the living structure and of its relations with other components.

In theory, cytologists, histologists, and pathologists recognize that different reagents may produce diverse types of coagulation products when applied to a given cell, and that due to possible distortions and abnormalities, the interpretation presents great difficulties. In actual practice, however, there is frequently a surprising disregard of those dangers. There are cytological papers, the conclusions of which are accorded important theoretical significance, in which the fixatives used are not even mentioned; the assumption is implicit that coagulation products in general are so dependable that it is unnecessary even to name the reagents. In many studies the fixatives employed are listed, but no mention is made of the specific one concerned in the case of each illustration; such procedure implies that the different types of coagulation products produced by the various fixatives all yield equally significant data for the living condition.

The extent to which cytologists ignore the different effects of various fixatives is apparent if one examines the descriptive matter accompanying the illustrations in cytological textbooks. In each case certain essential information is usually given—species, type of tissue, phase of cell history, and name of investigator; with rare exceptions, however, the fixative is not mentioned. This practice is difficult to explain, since it is universally admitted that in the case of many structures the detailed morphology may be radically modified by different reagents. "It is, I think, one of the most remarkable facts in the history of biological science that the urgency and priority of this question should have appealed to so few minds." This statement of Hardy's is as true to-day as when he wrote it in 1899.

The usual uncritical interpretation of coagulation products has produced in many quarters a general distrust of the cytological technique as a whole—a distrust that is in many respects well founded. It is as unscientific, however, to maintain that all coagulation products are largely undependable as to hold that they are all trustworthy. The situation differs widely in the case of different cell components.

When the coagulation products of a given component can be compared with the living condition, there is little difficulty in deciding which fixation images are dependable. For example, when Bělař's ('27) photographs of living grasshopper spermatocytes are compared with those of the same cells after fixation, it is obvious that the coagulated cells give accurate information concerning the number and relative sizes and shapes of the chromosomes. The coagulation products of plastids and other components can similarly be compared with the living structures in some cells.

In cases where the component cannot be seen in the living cell, the dependability of its coagulation products is determined with relative certainty if many different reagents produce similar fixation images when applied to the same cell type. For example, even if chromosomes were invisible in all living cells, their coagulation products would yield valid evidence as to their gross structure and changes in position because of the similarity of their fixation images in a given cell type, after the application of almost any common reagent. Coagulation products showing such constancy of structure can be explained only by the assumption that they are a relatively accurate fixation image of living components. Such dependability of the fixation products of chromosomes underlies the major contribution of cytology, i.e., the present theories concerning the physical basis of heredity.

In contrast to the above are those components which cannot be studied in the living condition, and whose coagulation products show extensive variability after the use of different reagents. In such cases it is very difficult to decide which classes of fixation images give dependable information concerning the living component. Zirkle's ('28, '29) photographs of corn root tips and Fry's ('29 b) drawings of metaphase asters in echinoderm eggs are examples showing the extent to which various fixatives, when applied to the same kind of cells, may produce a diverse array of coagulation products, many of which, owing to their very diversity, must be artifacts of the living structure. Such diversity of structure of fixed central bodies, for example, is largely responsible for the conflicting observations and hypotheses concerning them. The same situation renders difficult the study of chondriosomes and Golgi bodies.

2. The necessity for studying fixed cells

It is clearly recognized, as a general principle, that if information concerning a component's structure can be secured from a study of 'normal' living cells it usually constitutes primary evidence, in contrast to that obtained from coagulated cells, which is secondary. The investigation of living cells should be pursued by every available technique, whether it be tissue culture, microdissection, the application of photography, or other means, but the results must be viewed with proper scepticism. The very conditions of the experiment, such as the act of teasing apart the cells of tissues so that they can be studied, or the effects of vital dyes, may cause subnormal conditions and thereby produce distortions and abnormalities. A supplementary investigation of fixed cells may prove valuable.

When very transparent living cells are studied, such as those in tissue-culture preparations or transparent eggs under compression, the appearance of a living component is not necessarily the final and conclusive evidence as to its actual detailed structure. For example, although spindles appear to be structureless when alive, there is considerable evidence that in reality they have a differentiated, linear organization; an analysis of fixed spindles may yield valuable data on this point.

It is obvious that although the distinct visibility of a component in the living condition yields positive evidence concerning its structure, its invisibility, on the other hand, proves nothing. A structure may be present even in very transparent cells and yet be invisible because of similarities between its refractive index and that of the surrounding material. A study of fixed cells may produce valuable evidence in such a situation.

Those who have worked with living cells know how little can be seen distinctly in most of them. Many are so opaque that even when isolated the only method now available for studying their structures is through the use of fixed and sectioned material. In cells which are more or less transparent, the gross appearance of a component may be visible in the living state, but its detailed structure is frequently too vague to be analyzed accurately. For example, the radial organization of the asters is vaguely visible in some living cells, but the exact details cannot be ascertained; study of fixed asters may yield additional information. Again, a component may be readily studied in the living condition during one phase of its history, but this may be impossible at other stages. For example, in some cells the chromatin can be studied in the living state during mitosis when it occurs in the form of chromosomes, but its structure cannot be determined during the vegetative phase, and a study of fixed material may be the only source of information.

A QUANTITATIVE CYTOLOGICAL METHOD

The proposed method of studying fixed cells, outlined below, is designed to secure data concerning a cell component under the most difficult circumstances: when the cells cannot be studied in the living condition, and it is therefore impossible to compare the coagulated component with the living one; and when the component's coagulation products produced by different fixatives show great diversity of structure. In the case of cytological problems not presenting such difficulties the method can be greatly shortened and simplified.

Its essentials, however, are significant for all studies of fixed cells and tissues.

Many of the details of the proposed method are colored by the fact that it was developed in connection with a study of the mitotic mechanism of fertilized echinoderm eggs. Such cells present certain specialized conditions which do not occur in many investigations. The eggs are very large, having a diameter of about 100 μ; they are rapidly undergoing changes; most of them are in the same stage at any given time; they are separate from each other and not aggregated into tissues; their large division figure is highly differentiated. In order to make the presentation of the method concrete, it is described as it was actually applied to the study of such eggs. Every effort has been made, however, to give the discussion wide applicability to cytological studies in general.

1. Securing the data

Experiment A. Stage of cell cycle, fixation, stain, environment, and other factors—all constant. In the initial experiment all the factors are as constant as possible. The cells selected for study are all in approximately the same stage, either a given period of the vegetative condition or one of the mitotic phases of the cell type in question. Any common fixative is used; various reasons, such as its previous use in a similar study, may determine its selection, but it is largely immaterial which reagent is selected first, since others are employed later. The cells are sectioned at the proper number of micra; they are stained with one of the common dyes, such as Heidenhain's haematoxylin. They are in a 'normal' environment prior to fixation. The analysis is made in the following manner:

1. A sufficiently large number of cells to constitute an adequate random sample must be studied. The more numerous the variables and the wider their modifications, the larger should be the number analyzed. For example, in the initial study of fertilized echinoderm eggs (Fry, '29 a, etc.) a hun-

dred cells were examined at each stage: in later portions of the investigation, about twenty-five cells were considered a sufficient number.

2. The cells must be selected in such a way as to avoid any unconscious selection of certain classes within the stage under investigation. This is accomplished if study of the slide begins at one end, and a given area is completely and systematically covered by the aid of a mechanical stage so that without exception every cell in that area at that stage is noted until the necessary number is reached. Such procedure automatically includes all phases of the stage in question; hence the various modifications of the different variables are analyzed, including transition conditions between preceding and succeeding stages.

3. Each cell is measured and analyzed in the greatest possible detail, not only with reference to every point immediately concerning the component in question, but also with regard to all surrounding structures, since they may be directly or indirectly concerned. A tabular form is used for this analysis upon which every cell is checked with reference to every point. Unless such a form is employed, omissions inevitably occur when checking large numbers of cells with respect to many variables. The compilation of the list is of greatest importance; it must be as detailed and comprehensive as possible. For example, when cleavage figures are studied, each cell is checked with reference to the following points. *a*) Asters: dimension, in a line parallel with the spindle axis; in a line at right angles to the spindle axis; whether or not the ray area is differentiated into zones. *b*) Rays: whether scarcely organized, or faint, or distinct; whether delicate or coarse; whether relatively straight or crooked; whether having the form of a continuous line or a series of granules; whether equally long and of similar physical structure in the various regions of the aster. *c*) Spindle: length and width; structure of its fibers in contrast to that of astral rays. *d*) Centrosome, i.e., large diffuse type of central body: dimension, in a line parallel with the spindle axis; in a line

at right angles to the spindle axis; physical structure, whether granular (homogeneous or scattered), vacuolar, or 'empty,' etc.; whether or not traversed by rays; whether or not clearly demarked from surrounding ray area. *e*) Centriole, i.e., minute period-like type of central body: number; size; physical structure; exact location within centrosome; whether or not the situation in one aster of the mitotic figure is the same as that in the other; comparison of centrioles with cytoplasmic granules. *f*) Cytoplasmic granules: number and size in a given measured area at inner portion of rays; at outer portion of rays; and in the cytoplasm. *g*) Chromatin: exact details as to physical structure; location exactly measured with reference to the other components.

4. If the cell is cut in several sections, all the serial sections must be studied. In the case of very large components, such as asters of early cleavage, a section passing through the mid-region has a very different structure from a section of the peripheral area. In cases where a section is missing the cells involved are not analyzed, as their data would be incomplete.

5. When making measurements and noting the interrelations of various structures, it is necessary to know the plane in which the cell is sectioned. Since components rarely have spherical symmetry, either as to their structure or as to their relations with other components, sections cut in various planes have different appearances and measurements.

6. Cells on the same slide may differ considerably as to the depth of the stain. This is immaterial with reference to many points in the analysis, but may be a very important factor with regard to others. For example, the center of an aster, if deeply stained, is very dark and its details are obscured, owing to the convergence of astral rays which take up the dye; whereas, if it is lightly stained, the physical structure of the astral center is visible.

Experiment B. Fixation varied; stage of cell cycle, environment, stain, and other factors constant. Having made an initial study with all factors constant, thereafter follows a group of similar studies of the same stage, varying only the fixative. The same detailed method of analysis is used in the case of each type of coagulation product produced by each of a number of diverse reagents. The fixatives selected include: 1) those used in previous similar studies; 2) other common fixatives as well as such special ones as those designed to demonstrate chondriosomes and Golgi bodies; 3) the components of the fixing fluids used separately; 4) and, finally, various unusual reagents to secure coagulation products that may be of special significance. In some cases it may be desirable to study the effects of various lengths of time of fixation.

Experiment C. Stage of cell cycle varied; fixation, stain, environment, and other factors constant. After ascertaining the effects of various fixatives upon the structures during any given stage, the next step is a similar study of the different stages in the cell cycle. At first that fixative is used for each stage which was employed in the case of the stage studied above (experiment A). Thereafter other fixatives are applied to each of the various stages; their various coagulation products are analyzed in the manner previously described.

Experiment D. Environment varied; stage, fixation, stain, and other factors constant. It may be profitable to study in a similar way the effects of various modifications of different environmental agents, such as the hydrogen-ion concentration, nutrients, temperature, osmotic pressure, x-rays, and other factors, in order to determine how they modify cell structures prior to fixation. In the case of each environmental modification at each stage, after a given fixation, the same detailed analysis is made.

Experiment E. Stain or other factors varied; stage, fixation, and environment constant. It may be desirable to analyze the effects of different stains upon the component in question, including such special ones as the Fuelgen reaction, which is specific for certain substances. It may be profitable to study the effects of various depths of staining. If the cells are aggregated in tissues, it may be essential to compare the coagulation products of those near the edge with those in the interior. Significant results may be secured if a component is comparatively studied at various phases in the history of the organism, i.e., in spermatogenesis, oogenesis, fertilization, early cleavage, late cleavage, and in various adult somatic tissues. In some cases it may be necessary to determine whether or not various modifications of the running-up technique may modify the results.

2. Analyzing the data

The cells which have been measured and analyzed under varying conditions as described above are grouped in classes. Only those cells are classed together in which all the variables are similar in physical structure, size, and position. Cells showing significant differences in but one of the components are grouped separately. Hence transition classes occurring in small numbers, although possibly significant, are not overlooked. For each class there is ascertained its percentage in relation to the total number of cells studied under the same condition.

A preliminary sketch is made of each class, no matter how small or how large its percentage of the total. Each drawing illustrates not a single 'best' cell, but an entire class, all the members of which are similar in all respects. Cells that deviate in any significant manner, even slightly, from a given class are represented by a separate sketch. Needless to state, no attempt is made to illustrate averages of even minor modifications of physical structure; every significant variation, without exception, is shown in a separate drawing. Averages are used, however, in the case of certain dimensions; for example, the length of the spindle in any drawing is the average of the individual measurements made of spindle length in each cell of that group. Below the sketch of each class is noted its percentage of the total number of cells studied under the same condition.

This procedure visualizes for the investigator the changes undergone by each component in physical structure, size, and position, as they are modified by various fixatives, astral stages, and other factors. There is also apparent at a glance the relative percentage included in each class and the manner in which changes undergone by one component may be related to changes in others. With such drawings of all the coagulation products before him, the investigator decides which are the most accurate representations of the living condition. The method necessitates interpretation of the data, as cytological studies always do, but it places at the disposal of the student a more complete and quantitative body of observations than is secured by the usual practice.

The procedure makes possible the application of certain statistical methods to cytological research, since the relative percentage of each modification of each component in each class is known under each of many conditions. The exact measurements make feasible an analysis of dimensions, volumes, changes in position, and similar points.

3. *Determining living cell structures from their coagulation products*

It must be kept in mind that this discussion applies primarily to the study of those numerous cases where it is impracticable or impossible to observe the component alive, and where its coagulation products show considerable variability of structure. Hence the method concerns components such as central bodies and Golgi bodies, rather than those like chromosomes and plastids.

The proposed method is not designed to secure information concerning the chemistry of coagulation, although incidental observations in this field may occasionally be made, since many fixing fluids are used. Certain investigations (for example, Zirkle, '28, '29) have as a major purpose an analysis of the physical chemistry of coagulation, a problem of great significance. At present, however, such knowledge is scant and interpretation of the data is difficult, owing to the complexity of protoplasm. The present method assumes that even though nothing may be known about the chemistry of the coagulation products of any given component, those products can, nevertheless, be used to give dependable information concerning the morphology of the living condition. An analogy may clarify this point of view. Assume that one is called upon to make a drawing of a totally unfamiliar organism from a group of photographs, some vague, others developed only in certain places, others with wrinkled films, and others having distortions resulting from the use of faulty lenses. Some of them may be accurate, but there is no way of knowing this. By a careful analysis and comparison of each part of each photograph, it may be possible accurately to reconstruct the original. A knowledge of the chemistry of the photographic processes involved is not essential to making such a reconstruction. Similarly, by analysis and comparison of a number of fixation images each of which may possibly deviate from the original in various respects, it may be possible accurately to reconstruct the living condition. Information concerning the chemical composition of the coagulation products, although valuable, is not essential for reconstructing the morphology of the living component.

In all branches of biological science, a given technique applied to a certain material yields a more significant result for the problem in question than do other methods and materials. In cytological studies certain cells when coagulated by specific reagents are capable of producing fixation images especially significant for elucidating a given point, and such coagulation products are legitimately regarded as 'favorable.' The more variables there are in a given experiment, however, the more complete and exhaustive must be the study before one is able to select that situation which produces significant conclusions. If slight modifications of the technique modify the result, the greatest precautions must be exercised to ascertain their various effects, in order that they may be accurately taken into consideration.

The cytologist is well aware that in the case of many components the fixatives employed modify radically the coagulation products. Not only are such differences produced by different reagents, but they sometimes occur even in different sets of the same type of cell, fixed and run up at various times in supposedly the same manner; they are due to the effects of slight modifications of the process difficult to control exactly. It would be hard to name a branch of biology in which the technique causes wider diversity in the results than is the case in cytology, and in which there is consequently greater need for an exhaustive consideration of all possible effects of the method.

Despite the dangers inherent in the variabilities produced by fixation, the cytologist usually fails to use those simple quantitative methods which are readily applicable to his material, and which would help to avoid those dangers. Even in the case of components where it is known that different reagents modify the coagulated structures, he usually employs but one or a few fixatives, and fails to determine the relative percentages of the various classes within their coagulation products. Such a non-quantitative analysis yields a correct result in the case of components like chromosomes, where almost any common reagent gives a dependable fixation image; however, when coagulation products are radically modified by the reagents, such an incomplete and non-quantitative study may lead to serious errors, since the supposedly significant or 'normal' classes are chosen on the basis of incomplete and partial data.

The current method and the one here described have the common purpose of determining which classes of coagulation products are 'normal.' As previously noted, the methods differ as to the completeness with which they secure and analyze coagulation data. They differ also as to how early in the investigation certain classes of fixation images are determined as 'normal.' The accepted method decides too early in the study which coagulation products are 'normal' and devotes too much attention to them; meanwhile it too easily passes by others on the same slide as 'abnormal.' The proposed method regards all coagulation products, throughout the entire investigation, as of possible equal significance for arriving at the result, studying them all with equal intensity, and not until after an exhaustive study has been completed and the conclusion has been reached are any of the fixation images determined as 'normal' or significant or favorable.

At a given stage, after using various fixatives, the larger the percentage of a given class of coagulation products with reference to the total number of cells studied, the more probable is it that that class has significance for the living condition. The extent to which coagulation products are modified by various reagents differs widely with different components. In fertilized echinoderm eggs at the metaphase stage, for example, after the use of many common reagents, chromosomes show least modification, spindles are modified to a greater extent, asters show still more variability, while central bodies exhibit maximum diversity in structure. The fact that a certain type of coagulation product occurs in large numbers after the use of many reagents does not constitute proof that it is necessarily the one to be given most consideration. It is possible that a given component at a certain stage might be properly fixed in but one or several reagents out of many; hence the significant coagulation product would occur in but a limited percentage of the total number of cells analyzed. The smaller the percentage, however, the more necessary is it to secure evidence that it has meaning for the living condition.

No matter how small or how large the percentage of a certain type of coagulation product at a given stage after using various fixatives, if it is to be considered significant for the living condition it must be very similar in most of the cells in question. Any coagulated structure that shows wide diversity as to form, location, physical appearance, or number, especially when such diversity occurs within the coagulation products of but one fixative, must be viewed with great scepticism. If such a variable structure is not quantitatively analyzed, it is possible for the investigator to select any one of the many modifications as 'normal,' meanwhile dismissing the others as 'abnormal.' For example, after fixation with most reagents, small black granules are abundant throughout the cytoplasm of echinoderm eggs. They occur sporadically at the centers of the asters, differing as to size, number, and exact location. The selection of certain granule configurations occurring in very limited numbers as 'normal,' coupled with a dismissal of the majority of the configurations as 'poorly fixed,' has led to their interpretation as centrioles (Fry, '28 a, '29 b). To avoid errors of this type, it is essential that there be known in relation to the total number of cells studied, the exact percentage of each type of coagulation product of each component, at each stage in the cell history, after fixation with many reagents. All of the facts must be taken into consideration in arriving at the result.

It is to be remembered, however, that no matter how completely and quantitatively coagulation products are studied, if they cannot be compared with the living component and if they show extensive variability after the use of different reagents, conclusions must be drawn with great caution. There is more probability, however, of arriving at a valid result if a wide variety of fixation images are quantitatively considered than if the usual procedure is followed.

The proposed method has been criticized as being interested in 'quantity' rather than in 'quality'; it has been described as studying superficially a wide variety of many fixed cells, rather than concentrating upon the study of a few 'best' cells. Such criticisms are based on a misunderstanding of the method. Assume that one selects any previous important cytological paper, the results of which are admittedly based on a careful study of 'best' cells according to the usual standards, and assume that but one fixative was employed. A reexamination by the method here described of the same kind of cells fixed by that same reagent would be more complete and accurate than was the previous work, because of the systematic way in which cells are measured and analyzed in large numbers, selected at random at all intervals. This, however, would be only the first stage of the new investigation. After this initial phase of the work there would follow an equally detailed analysis of coagulation products in each of a number of experiments that differ from each other in fixation or other factors. The proposed method is interested in 'quality' as well as in 'quantity.' In order to determine which ones are 'best,' it studies in an equally detailed manner many different types of coagulated cells rather than confining attention to the supposedly 'best' cells selected too early in the investigation upon insufficient evidence.

OLD AND NEW HYPOTHESES CONCERNING THE MITOTIC FIGURE OF ECHINODERM EGGS DEVELOPED BY THE OLD AND NEW METHODS

The proposed method is spoken of as new. It is new, however, only in the organization of its details; the elements of which it is composed are old. For example, the dangers of misinterpreting coagulation artifacts have been repeatedly discussed in the past, as noted above (p. 1). The new method stresses the fact that even though coagulation products may be distortions of the living condition, nevertheless, if properly analyzed they can be used to secure valid information concerning cell structures. Furthermore, there have been sporadic efforts from time to time to apply simple statistical methods to cytological study, by ascertaining the various percentages of different coagulation products. Pollister ('29) has called the author's attention to such studies of central bodies made by Heidenhain in lymphocytes and by Ballowitz in epithelial cells. Such investigations may be of little significance, however, if the coagulation products of only one or a few reagents are so analyzed. The proposed method emphasizes the necessity of studying quantitatively the coagulation products of many diverse reagents. Again, various cytological studies have made careful measurements of cell structures, as in Conklin's ('12) analysis of developing Crepidula eggs. But the essential fact remains that, no matter how many attempts have been made in the past to place the cytological technique on a scientific, objective, and quantitative basis, those attempts have failed to modify the general practice. The current cytological method fails, despite all warnings, to guard against the misinterpretation of coagulation artifacts.

An illuminating way to emphasize the differences between the current method and the one here described is to contrast the conclusions reached when the two techniques are applied to the study of the mitotic mechanism of fertilized echinoderm eggs. The eggs of Echinarachnius parma have been analyzed by the proposed method; the conclusions differ radically from those of previous investigations of echinoderm eggs made by the usual method. The contrasts between the old and new conclusions are here briefly outlined, on the assumption that the extent to which they differ from each other is a probable measure of the respective validity of the two methods of analyzing coagulation products.

These more recent results indicate that in fertilized Echinarachnius eggs, which are probably typical of echinoderms generally, the so-called central bodies, which are assumed to be the formative foci of the mitotic figure and its most persistent element, actually have no existence in the living egg. The usual hypothesis in the case of echinoderms is probably based on two fallacies. First, cytoplasmic granules, abundant throughout the cytoplasm, occasionally have a random distribution at the astral mid-region. Certain configurations occurring sporadically have been misinterpreted as centrioles, i.e., the small period-like type of central body. Secondly, when rays are well formed and extend to the aster's central area, fixation produces a differentiated coagulation artifact at the focal region. This has been misinterpreted as a centrosome, i.e., the large diffuse type of central body (Fry, '28 a, '29 a, '29 b; Fry, Jacobs, and Lieb, '29). This so-called centrosome is always absent if rays are not well organized or if they are well formed peripherally, but fail to extend to the midregion; it arises only when well-formed rays arise, and always disappears when they fade; it changes size and shape as the aster changes size and shape; its structure undergoes radical changes during the astral cycle. The demonstration of this relationship is a result of analyzing quantitatively many classes of coagulation products, under various conditions of fixation, astral stage, and environmental factors; all types, without exception, were studied with equal care; they were all regarded as possibly equally significant.

The present result concerning central bodies is based on the study of cleavage figures in an echinoderm egg. It has been confirmed, with minor modifications, in first cleavage figures of the nemertine, Cerebratulus (Fry, '29 d), and also in maturation figures of Asterias eggs (investigation in progress). The extent to which the conclusion applies to central bodies in other animal cells remains to be seen, but since the new results have been established in cells heretofore assumed to have typical central bodies, at least some of the usually accepted facts have been called into doubt. The question immediately arises: what is the significance of the newer result with regard to central bodies of spermatocytes? In answer to this the suggestion is made that central bodies of spermatocytes may have less to do with the mitotic figure than is generally assumed; they are primarily blepharoplasts of the sperm's axial filament, perhaps playing no active rôle in mitosis. In favor of this suggestion is the fact that such structures are present in higher plants only in male motile gametes; they are absent in seed plants where the male gamete is non-motile. In any event, it is unsafe to reason from the very specialized behavior of components during spermatogenesis to their behavior in cells generally.

The newer studies have also resulted in certain significant observations concerning asters and spindles, owing to the fact that cells are chosen at random at every interval and each component is accurately measured. 1) In Chaetopterus eggs the sperm aster does not divide to form the first cleavage figure, as previously described by Mead, but the sperm aster, after elongating, completely disappears; the cleavage figure actually arises as a new structure (study in progress). This result is in harmony with Fry's description of the origin of the first amphiaster in Echinarachnius eggs ('28 b), but is contrary to the condition described by Wilson in the related form Toxopneustes. The problem of the origin of amphiasters with reference to preexisting asters is in confusion; further study of the subject may bring the facts under one category and simplify an eventual explanation of the process. Differences in species, however, may account for the discrepancies. 2) In both Chaetopterus and Asterias eggs the dimensions of the spindle and asters in the second polar body figure are exactly one-half those of the first. It is widely recognized that second polar body figures are usually smaller than the first, but because of failure to secure accurate measurements in most studies the exact degree of the difference has been determined only rarely. The question arises: is the ratio of 2:1 with respect to the dimensions of first and second polar body figures related to the same ratio with respect to the diploid and haploid chromosome groups? 3) In Echinarachnius eggs, during early development as far as gastrulation, the most constant cell measurement is the diameter of the metaphase chromosome plate, which determines the width of the spindle. This is reduced almost imperceptibly during early cleavage, although the cell volume is reduced five hundred fold (Fry, '29 c); hence the chromosome group appears to be that factor which undergoes least volumetric change during segmentation in Echinarachnius eggs. If components in cleaving eggs of various species were accurately measured, as was done in the present study, and in Conklin's investigation of Crepidula eggs, various significant relationships would be made apparent. 4) In none of the mitotic figures studied, whether in oogenesis or in cleavage, do the spindles elongate, except to a very slight and insignificant degree. In these cases the movement of the chromosomes cannot be related to spindle elongation—a relationship which has been demonstrated in some other cells, as in Bělař's studies of grasshopper spermatocytes. 5) During Echinarachnius development the asters disappear completely during gastrulation—a situation that also occurs in Chaetopterus and Cerebratulus. Thereafter the mitotic figure is like that of the typical plant cell composed of a spindle only. Theories of mitosis in the animal cell are chiefly based on a study of eggs during first cleavage. The question suggests itself: is it legitimate, as is usually done, to regard the mitotic figure of blastomeres with their giant size as typical of animal cells generally, especially since in some cases asters and central bodies completely disappear during early development as the cells become smaller?

The manner in which the new body of observations raises doubts concerning various phases of current theories of mitosis is another illustration of the obvious fact that a valid physiological theory concerning a given structure is dependent upon an accurate morphological description of that structure. When conclusions drawn from incomplete or inaccurate morphological observations are built into a physiological theory, the result is necessarily undependable. Any eventual formulation of an adequate physicochemical explanation of mitosis will require, among other things, a more accurate body of knowledge concerning the morphology of mitosis than is now available.

It is hoped that the proposed method may place the interpretation of coagulated cells upon a more scientific basis than does the current procedure. It is possible that its application may prove fruitful in various fields of cytology and histology. It is likewise possible that a cytological analysis of pathological tissues by the proposed technique will prove valuable in various phases of that field.

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ON THE DEVELOPMENT OF THE NERVES TO THE MAMMALIAN HEART

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THREE PLATES (NINE FIGURES)

This paper records the results of an attempt to find an embryological key to the bewildering maze of nerves to the mammalian heart.

Of the very extensive literature on the cardiac nervous system, four papers have been found very helpful. First, there is Hirschfeld's ('50) description of the gross anatomy of the nerves to the human heart. Perman ('24) has recently made a more penetrating study covering man and domestic animals. His paper is invaluable to every student of heart structure and function. Completing the macroscopic studies of Hirschfeld and Perman is the painstaking investigation on the distribution and connections of the ganglia of the human heart made by Francillon ('28). The story of the development of the nervous system of the heart begins with the classic work of His, Jr. ('93)—a paper which is all the more to be admired when one remembers the state of embryological technic and of nerve-impregnation methods of the time. Perman ('24), in addition to his gross anatomical work, has studied Bielschowsky preparations of embryonic chick and mammalian hearts, and has opened the way for a new attack on His's problem with modern methods.

The study here reported is based on hearts of calf embryos ranging from 14 mm. to 100 mm., i.e., from the time of the first appearance of heart nerves and of the first traces of the atrioventricular node to the time when the sino-atrial node can be recognized. All hearts were prepared by the Bielschowsky method, as modified by Agduhr ('17). Graphic reconstructions of the nerves were made from each successful preparation, the nerves being plotted upon heart outlines that were based on wax models or fresh hearts. Since few series were perfectly impregnated, the stages and figures to be described below are mostly composite pictures derived from a number of series and reconstructions.

Despite Agduhr's lucid and comprehensive directions, many difficulties cropped up in the preparation of material which had to be in large pieces and which included much connective tissue and cardiac muscle, two refractory tissues for the Bielschowsky method. The writer is deeply indebted to Mr. A. G. Fairall, whose technical knowledge and resource make this paper possible.

CARDIAC NERVES IN EMBRYOS OF UP TO 30 MM.

The first traceable branch of the vagus nerve in the heart region is the inferior laryngeal nerve, which hooks around the pulmonary arterial arch in a 12-mm. embryo. In a slightly older 14-mm. embryo (fig. 1) three recurrent branches of the vagus can be traced, each running behind one of the last three arterial arches to end on the near-by pharynx. The lowermost is the familiar inferior laryngeal nerve of adult anatomy. The middle branch hooks around the fourth or aortic arterial arch. It corresponds to the recurrent oesophageal nerve of some reptiles (Van Bemmelen, '88). This middle branch seems to be vestigial in mammals. The uppermost nerve hooks around the third or carotid arch. The atrophy of the dorsal aorta permits the nerve to escape and to become the ordinary superior laryngeal nerve. It should be noted in passing that the nodosal ganglion is strung out along the vagus nerve and ends in a special heaping of cells about the origin of the inferior laryngeal nerve. Another peculiarity of the vagus of the calf at this and all later stages is the presence of a commissure that connects the two nerves and runs across the ventral surface of the oesophagus just below the heart (figs. 1, 2, 3). From the commissure springs a median ventral vagus trunk.

The first branch to the heart from the vagus nerve can be traced in a 14-mm. embryo. It leaves the vagus some distance below the inferior laryngeal nerve and ends on the surface of the common cardinal vein (fig. 1). This nerve, the most important of all nerves to the heart, can be named the nerve to the cardinal vein.

In embryos of around 20 mm. appear two more vagus branches for the heart. They are shown in figure 2, a composite drawing based on six reconstructions. In addition to the nerve to the cardinal vein, there is on each side one stout twig to the pulmonary arteries and another to the bulbar end of the heart.

The right nerve to the common cardinal vein follows the dorsal margin of the anterior cardinal vein (fig. 2) to the level of the sino-atrial valve. Below this point the nerve follows the dorsomedial side of the common cardinal vein on to the right horn of the sinus venosus. There the nerve lies behind the left valve leaflet and the interseptovalvular space. In the same fashion the left nerve to the cardinal vein runs along the dorsal and dorsomedial sides of the left anterior cardinal vein on to the left horn of the sinus venosus. Twigs from both branches run to adjacent heart muscle. The branches shown in figure 2 are possibly fragments of a continuous plexus which extends across the sinus venosus from one nerve to the other.

The nerve to the pulmonary arteries in the 18-mm. embryo arises on each side just above the nerve to the cardinal vein and below the inferior laryngeal. The two nerves proceed directly to the corresponding pulmonary arteries, where they break up into a common plexus that envelops both vessels. Traces of the nerve to the pulmonary artery can be found in the earlier (14-mm.) embryo.

In a few series a nerve or pair of nerves to the bulbar end of the heart can be followed. They are quite clear in older embryos. Each 'bulbar' nerve, as this twig can be named, arises above the inferior laryngeal, not below it, and runs to the space between the ductus arteriosus and the aortic arch.

In addition to the three pairs of nerves to the heart, three definite plexuses can be traced. The plexus over the sinus venosus in which the nerves to the cardinal vein end and the plexus around the pulmonary arteries supplied by the nerves to the pulmonary arteries have already been referred to. Below the fairly coarse plexus around the pulmonary arteries is a finer one surrounding the pulmonary vein and spread out over the adjacent atrial wall. This plexus around the pulmonary vein is supplied by twigs from the other two plexuses and by a special twig from the ventral vagus trunk just below the commissure.

In general, the effort to trace the development of the sympathetic innervation of the heart ended in failure. The sympathetic trunk of the 14-mm. embryo is everywhere distinct from the vagus. In a 20-mm. embryo the two are fused from the base of the skull to the ansa subclavia and stellate ganglion (as in fig. 3). In many embryos of this period there are traces of sympathetic twigs that follow the common cardinal veins to the heart. It should be noted that the vagus branches to the heart arise below the fusion of the sympathetic trunk and vagus nerve. In all likelihood, the vagus branches contain sympathetic components.

By the time the calf embryo has reached the C.R. length of 20 mm. the general plan of heart innervation is laid down. There are three pairs of vagus branches, to the cardinal veins, to the pulmonary arteries, and to the bulbar end of the heart. The nerves probably appear in the order named. To these may be added distinct sympathetic filaments to the sinus region.

CARDIAC NERVES IN EMBRYOS OF FROM 30 TO 40 MM.

In embryos of from 30 to 40 mm. the chief advances are to be found in the nerves to the cardinal veins and in the plexus over the sinus venosus.

The nerve to the right cardinal vein (figs. 3 and 5) runs down the dorsal side of the vein until the vein is about to plunge into the sinus venosus. At that point a median twig is given off which breaks up into a tiny plexus over the site of the future sino-atrial node, and the interatrial muscle band of Papez ('20). The rest of the nerve continues down over the right horn of the sinus venosus, and divides into two terminal branches which follow the line of attachment of the two leaflets of the sino-atrial valve (figs. 3 and 6). The lateral of these branches breaks up over the sinus and adjacent atrium. The medial branch ends in the general plexus of the sinus venosus. A stout twig (*x* in figs. 3 and 7) penetrates the connective tissue at the base of the atrial septum and gains the anterior atrial surface at the level of the aortic semilunar valves. There it ends in a peculiar node-like mass of tissue (fig. 7) which appears in all young calf embryos, and which I have described elsewhere as the 'inter-atrial node' (Shaner, '29).

The nerve to the left cardinal vein runs down along the vein to end in an elaborate plexus over the left horn of the sinus venosus. Definite twigs from the plexus penetrate beneath the atrial septum and end in the atrioventricular node (figs. 3 and 8). The innervation of the sino-atrial and inter-atrial nodes belongs definitely to the right nerve. That of the atrioventricular node belongs to one nerve as much as to the other.

Ganglion cells appear along the left nerve to the cardinal vein in a 37-mm. embryo. In a 40-mm. embryo they can also be found along the right nerve, and in the general plexus of the sinus (fig. 3).

The origins of the nerves to the cardinal veins and to the pulmonary arteries have moved up the vagus nearer to the inferior laryngeal nerve.

The plexuses around the pulmonary arteries and around the pulmonary vein are now more extensive. That around the arteries is distinctly coarser than that of the vein (figs. 3, 5, and 6). A plexus can also be traced between the aortic arch and ductus arteriosus at the ends of the two bulbar nerves.

Another new development is an extensive plexus encircling the atrio-ventricular groove (fig. 3). It appears to have spread from the plexus about the sinus venosus and to have extended forward on either side toward the roots of the aorta and pulmonary arteries.

A nerve from the ventral vagus can be traced to the plexus about the pulmonary vein as in earlier stages.

A determined effort to trace sympathetic branches to the venous end of the heart ended in general failure. In a slightly older 50-mm. embryo a stout sympathetic branch leaves the left ansa subclavia and ends on the brachiocephalic artery—the first great artery to leave the aorta in the calf. This nerve is conspicuous in older foetuses, where it extends downward on to the bulbar part of the heart (fig. 9).

CARDIAC NERVES IN EMBRYOS OF FROM 79 TO 100 MM.

The nerve supply of hearts from 79- to 100-mm. calf embryos is of special interest because of its relation to the sino-atrial node, which appears during this period. The node differentiates slowly and is first undoubted in a 100-mm. embryo (Shaner, '29).

On the superior vena cava (fig. 4) two closely associated nerves pass over from the trachea on to the vein, which together represent the right nerve to the cardinal vein of earlier stages. The medial nerve ends in two small ganglionated plexuses, the one over the site of the sino-atrial node, the other on the interatrial muscle band of Papez. The lateral nerve follows the vena cava superior down on to the sinus venosus and extends a short distance over the inferior vena cava. The nerve ends in a plexus which communicates with the plexuses about the pulmonary arteries and veins and with the plexus around the atrioventricular groove. The nerve supply to the inferior vena cava is scanty, as Francillon ('28) has already pointed out.

The left nerve to the left cardinal vein arises directly from the left inferior laryngeal nerve. It passes down the vein on to the left horn of the sinus venosus to break up into a richly ganglionated plexus that is continuous with a similar plexus originating from the right nerve.

Ganglionated twigs pass from the plexus into the atrioventricular node. No ganglia could be found within the node. The 'interatrial node' has disappeared at this age. Nerve cells all over the heart lie superficially enmeshed in the plexuses just beneath the visceral pericardium or contained in extensions of the subpericardial connective tissue into the atrial septum.

The two nerves to the pulmonary arteries run as before to the plexus around the arteries. The left nerve is very short, and comes off the inferior laryngeal nerve.

The plexuses around the pulmonary arteries and veins are heavily ganglionated. A twig from the ventral vagus trunk ends, as hitherto, in the plexus about the pulmonary veins. Extensions of the plexus around the veins enter the connective tissue of the atrial septum.

The two nerves to the bulbar end of the heart can be picked up along the brachiocephalic artery. The left nerve follows the artery. The right nerve swings around an extension of the pericardial cavity that pushes up along the artery—the 'aortic recess' of Perman. Both nerves reach the space between the aortic arch and the ductus arteriosus, and end there in extensive and heavily ganglionated plexuses, which can be traced down to the origin of the coronary arteries.

The plexus encircling the atrioventricular groove, which could be traced in the earlier embryos, is now more elaborate and is heavily ganglionated like the rest. On the posterior surface of the heart the plexus communicates with the plexus of the sinus venosus. On the anterior surface the atrioventricular plexus meets the plexuses that have extended down between the aorta and ductus arteriosus. The coronary plexuses of adult anatomy thus come into being.

I found it impracticable to map the nerve supply of hearts older than 80 mm. The large masses of tissue involved are difficult to impregnate by the Bielschowsky method and to cut into satisfactory serial sections. The advances of a 100-mm. embryo over the 79-mm. one described above lie altogether in greater detail, which becomes so great as to make mapping an exceedingly laborious undertaking.

ADULT BEEF HEART

The embryological story can be applied fairly well to the adult nervous system of the beef heart. The adult arrangement is given by Perman ('24), whose diagram is reproduced with slight alterations in figure 9. I have checked his diagram by independent dissections. Perman's work should be very useful to every student of heart physiology or anatomy who works with any laboratory animal.

Nerves 1 and 3 in Perman's diagram (fig. 9) are my nerves to the bulbar end of the heart. No. 1 is indeed a mixed nerve, but sympathetic elements cannot be ruled out of any of the embryonic nerves described above. The nerve as described by Perman has the same peculiar course around the 'aortic recess' that the right embryonic bulbar nerve follows.

Perman's nerves 4 on the left side and 5 on the right correspond to the embryonic left and right nerves to the cardinal veins. No. 4 is a mixed nerve, with its vagus component arising from the inferior laryngeal; no. 5 similarly has a vagus component.

Nerves 2 and 6 in Perman's diagram are sympathetic in make-up. Of these, no. 6 is the sympathetic nerve to the superior vena cava, that is indicated but not traceable in many embryonic series. Nerve no. 2 is the prominent nerve from the ansa subclavia which could be traced in 50-mm. embryos.

This leaves the two nerves to the pulmonary arteries still not accounted for. The left nerve must be very short and quickly lost in the maze of nerve fibers beneath the adult aortic arch.

APPLICATION TO THE HUMAN HEART

Caution must be exercised in applying the above data to the human heart. There are considerable differences between the hearts of the calf and man. Perhaps the chief application of the developmental story of the calf to the human heart is to throw emphasis upon the human inferior cardiac branches of the vagus. The inferior cardiac nerves are the equivalents of the embryonic nerves to the common cardinal veins. If embryology is any guide, the inferior cardiac nerves may have much to do with the nodes and conduction system of the heart. The usual text-book descriptions of the nerve supply to the human heart stress the superior cardiac nerves and the connection of all cardiac nerves with the plexuses beneath the aortic arch. This part of the nerve supply is more conspicuous in dissections, but not necessarily the more important for heart action.

The gradual development of the cardiac plexuses in the calf provides a sketch map to follow the more elaborate ramifications of the same plexuses in the human heart. A very careful study of the latter has recently been made by Francillon ('28). He pictures all cardiac plexuses as making up a more or less continuous plexus around the venous and bulbar orifices and around the atrioventricular groove. The plexus fibers do not extend for any distance over the ventricles. Francillon emphasizes the superficial position of the plexuses, that they lie everywhere in the connective tissue just beneath the pericardium or in slight extensions of the connective tissue into crevices in the muscle wall. Nerve fibers can be traced into the nodes, but nerve cells do not occur in them.

In the calf the extent of the plexuses is about the same. In the calf, as in man, all plexuses lie superficially, and do not penetrate into muscle masses. Nerve cells seem absent from the nodes.

DISCUSSION

The laborious job of ferreting out the nerves to embryo hearts cannot but increase one's admiration for the work of His, Jr. ('93), on the development of the nervous system in vertebrates. One hesitates to pick flaws in such a paper. I am forced to agree with Perman ('24), who like myself has the advantage of the Bielschowsky method, that His erred in placing the bulbar nerves ('Bulbusgeflecht') earlier in development than the nerves to the venous end of the heart ('Vorhofsgeflecht'). Perman considers that both sets of nerves arise simultaneously; my results would make the venous set appear slightly earlier. This brings the mammalian heart in line with the fish heart, where His found nerves to the venous end of the heart some time before nerves to the bulbar end appeared. In the earlier human embryos His finds a connecting plexus ('Verbindungsgeflecht'), between the venous and bulbar plexuses and nerves, which he follows to the adult deep aortic plexus. Whether this plexus corresponds to my nerves to the embryonic pulmonary arteries is hard to say.

SUMMARY

1. Three branches from each vagus nerve grow to the calf heart: *a*) a nerve to the common cardinal vein, *b*) a nerve to the pulmonary artery, and, *c*) a nerve to the ductus arteriosus and aorta. In addition, a twig from the ventral common vagus trunk approaches the heart from behind to end in a plexus around the pulmonary vein. The first-named nerve appears in 12-mm. embryos; all nerves are present in a 20-mm. embryo.

2. The earliest plexuses appear at the ends of the nerves. The terminal plexuses of the two nerves to the common cardinal veins spread over the sinus venosus. The terminal plexuses of the nerves to the pulmonary arteries surround the arteries. A finer plexus surrounds the pulmonary vein, and is fed by the surrounding plexuses and by the twig from the ventral vagus trunk. The terminal plexuses of the nerves

to the bulbar end of the heart extend downward to the origin of the coronary arteries. A plexus derived from that covering the sinus venosus encircles the atrioventricular groove, meeting the bulbar plexus in front.

3. A branch from the nerve to the right common cardinal vein ramifies at an early period over the site of the future sino-atrial node, and forms there a ganglionated plexus long before the node appears.

4. Twigs from the common plexus over the sinus venosus penetrate beneath the atrial septum to the atrioventricular node. The twigs belong to either nerve to the common cardinal veins.

5. Ganglion cells first appear on the plexus covering the sinus venosus in embryos of 37-mm. length. Cells appear soon afterward in other plexuses.

6. Sympathetic branches could not be traced with satisfaction. They seem to approach the heart, *a*) via the common cardinal veins to the sinus part of the heart and, *b*) via the brachiocephalic artery and aorta to the bulbar end of the heart.

7. From the standpoint of development, the inferior cardiac nerves and their connections with the plexuses around the venous orifices of the human heart are more important than the more conspicuous superior cardiac nerves and the plexuses beneath the aortic arch.

The writer is indebted to the Swift Canadian Company for the embryos used in this and other studies on the heart, and to Dr. D. G. Revell, who has kindly read the manuscript.

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ABBREVIATIONS

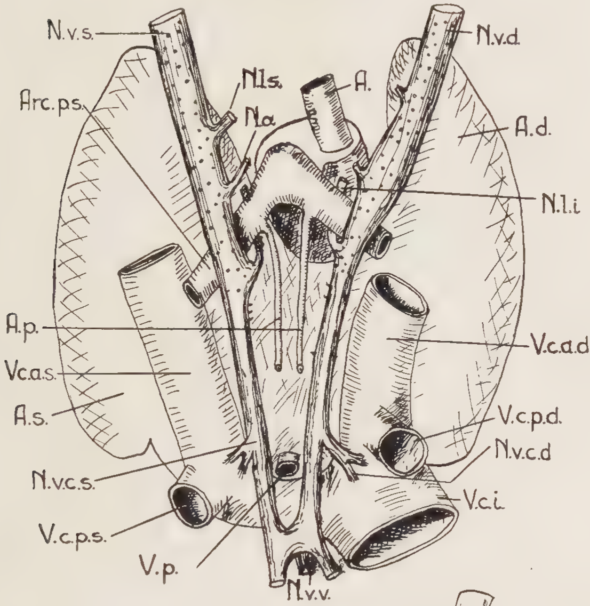
A., aorta
A.b., brachiocephalic artery
A.d., s., right and left atrium
An.s., ansa subclavia
A.p., pulmonary artery
Arc.p.d., s., right and left pulmonary arch
A.s.d., s., right and left subclavian artery
B.c., bulbus cordis
D.a., ductus arteriosus
N.a.p.d., s., right and left nerve to pulmonary artery
N.b.d., s., right and left nerve to bulbar end of heart
N.l.i., inferior laryngeal nerve
N.l.s., superior laryngeal nerve
N.o., recurrent oesophageal nerve
No.a-v., atrioventricular node
No.i-a., interatrial node
N.v.c.d., s., right and left nerve to the cardinal vein
N.v.d., s., right and left vagus nerve
N.v.c., nerve to heart from ventral vagus trunk
N.v.v., median ventral vagus trunk
N.s-v., nerve to sino-atrial node
P.a.p., plexus around pulmonary arteries
P.a-v., plexus in atrioventricular groove
P.s., plexus around sinus venosus and coronary sinus
P.v.p., plexus around pulmonary veins
S.v., sinus venosus
T.s., sympathetic trunk
V.c.a.d., s., right and left anterior cardinal vein
V.c.p.d., s., right and left posterior cardinal vein
V.c.i., vena cava inferior
V.d., s., right and left ventricles
V.p., pulmonary vein
x., nerve to interatrial node
y., nerve to atrioventricular node

PLATE 1

EXPLANATION OF FIGURES

1 Posterior view of heart of a 14-mm. calf embryo. Graphic reconstruction. × 15.

2 Posterior view of heart of an 18-mm. calf embryo. Graphic reconstruction. × 15.


N.v.s.
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N.v.d.
Arc.p.s.
N.a
A.d.
N.l.i
A.p.
V.c.a.d.
Vc.a.s.
A.s.
V.c.p.d.
N.v.c.d
N.v.c.s.
V.c.i.
V.c.p.s.
V.p.
N.v.v.

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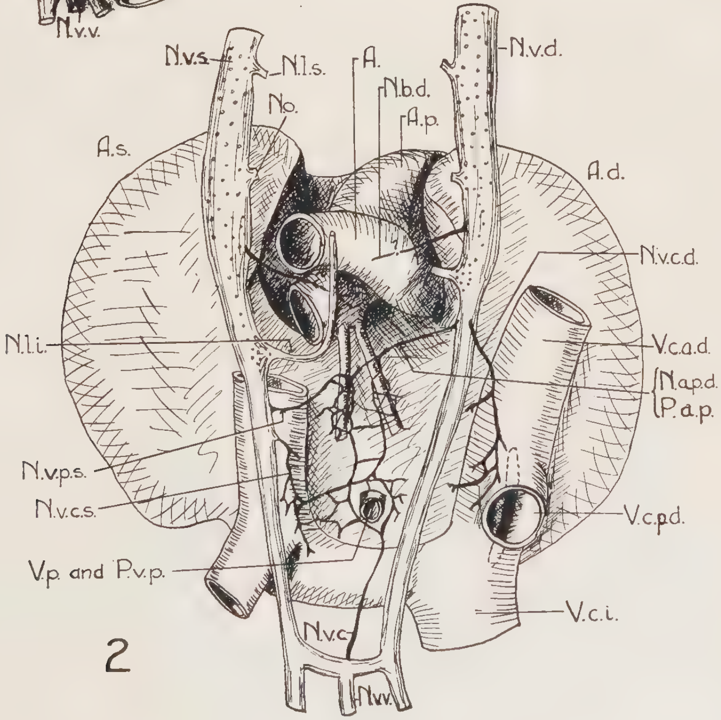
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N.l.i.
V.c.a.d.
N.a.p.d.
P.a.p.
N.v.p.s.
N.v.c.s.
V.c.p.d.
V.p. and P.v.p.
V.c.i.
N.v.c.
N.v.v.

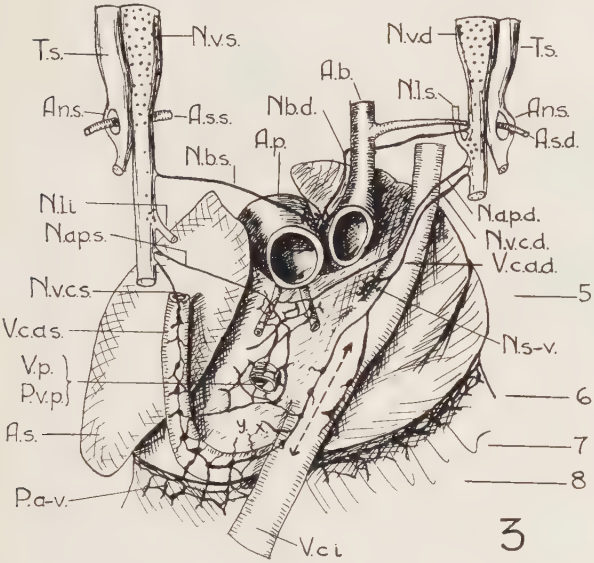
2

PLATE 2

EXPLANATION OF FIGURES

3 Posterior view of heart of a 40-mm. calf embryo. Graphic reconstruction. × 15.

4 Posterior view of heart of a 79-mm. calf embryo. Graphic reconstruction. × 12.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N.v.d
T.s.
A.n.s.
A.s.d.
N.l.i.
N.a.p.s.
N.a.p.d.
N.v.c.d.
V.c.a.d.
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V.c.a.s.
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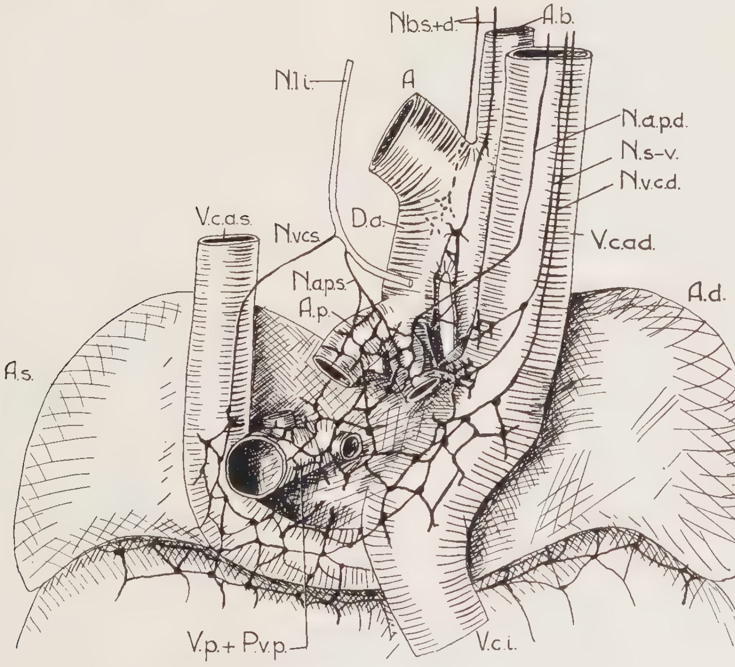
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N.v.c.s.
D.a.
V.c.a.d.
N.a.p.s.
A.p.
A.d.
A.s.
V.p.+P.v.p.
V.c.i.

4

PLATE 3

EXPLANATION OF FIGURES

5 to 8 Sections through the heart of a 40-mm. embryo, at levels indicated by lines in figure 3.

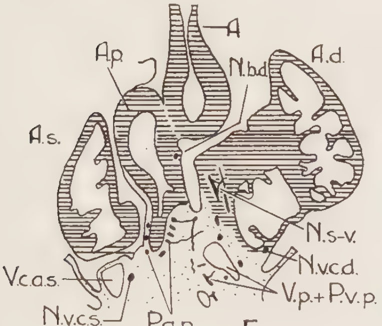
5 Section through site of sino-atrial node.

6 Section through pulmonary vein.

7 Section through interatrial node.

8 Section through atrioventricular node.

9 Diagram of the nerves to an adult beef heart. Slightly modified from Perman ('24).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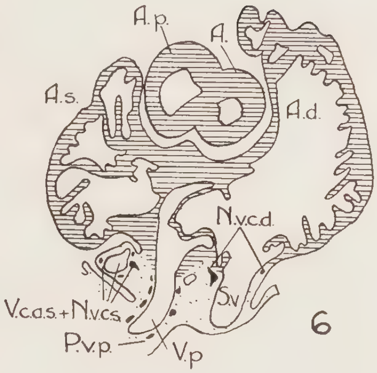
5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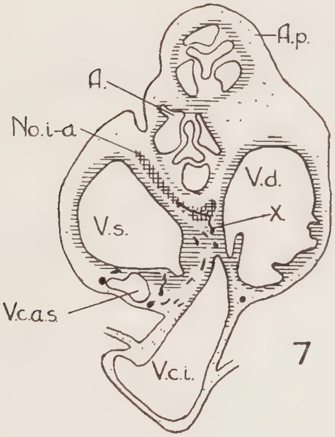
V.p

6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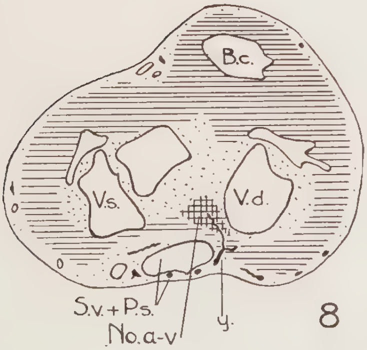
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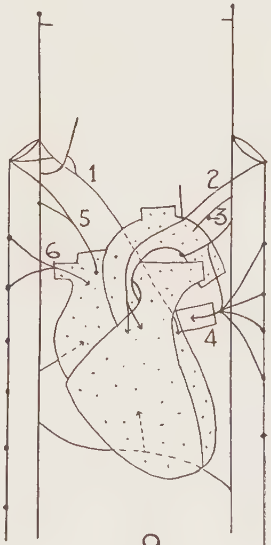
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9

DEVICE FOR PROLONGED CONTINUOUS CHANGE AND AGITATION OF FLUIDS

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Laboratory of Histology and Embryology, Western Reserve University

ONE FIGURE

The apparatus described here furnishes a simple, easily operated means of facilitating certain tedious processes of histological technique in which frequent change and agitation of the fluids are advantageous. In this laboratory it has been used notably for washing out pyridin with doubly distilled water in pyridin-silver methods of the Bielschowsky or Cajal type, where it considerably reduces the time necessary for the complete removal of pyridin and accomplishes this with the use of a minimum amount of water. It is also an aid in the decalcification of teeth and bone, and can be used for prolonged treatment in fixing fluids. Other applications in special situations will readily suggest themselves.

The device is simply an adaptation of the inverted-carboy type of drinking fountain frequently used in offices, or the water bottles used in animal cages. The set-up, shown in the figure, needs but little further explanation. It consists of a reservoir, which may be a large flask or bottle, fitted tightly with a one-hole stopper, and a delivery tube of an inside diameter of from 9 to 15 mm. The inserted end of the delivery tube should be flush with the deep end of the stopper and its distal end should be slightly beveled or notched. This assembly is filled to the stopper with the desired fluid and supported in an inverted position on a ring stand so that the distal end of the delivery tube lies at a level about 6 to 8 mm. below the rim of a small Stender dish. The fluid will flow into the Stender dish until it rises above and seals the outlet of the delivery tube. The specimen to be treated is placed in the Stender dish, supported on a few fine glass rods or beads strewn on the bottom. A capillary siphon (use a wick when particles which would clog a siphon are present) delivering at the desired rate is then hung over the edge of the Stender dish. The short arm of the siphon should lie at a level below that of the distal end of the delivery tube. When sufficient fluid has drained through the siphon, breaking of the surface film at the end of the delivery tube allows a large bubble of air to enter the reservoir, upon which a volume of fluid rushes from the end of the delivery tube, stirring the contents of the Stender dish. This process is repeated rhythmically as long as the supply of fluid in the reservoir lasts.

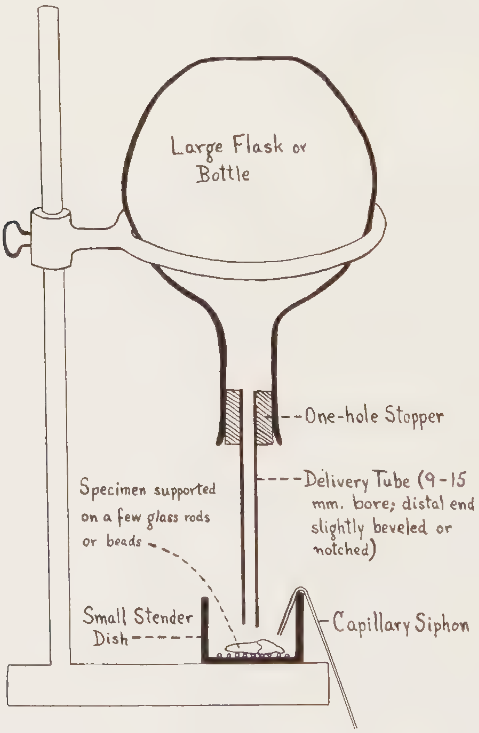
Large Flask or Bottle
One-hole Stopper
Specimen supported on a few glass rods or beads
Delivery Tube (9-15 mm. bore; distal end slightly beveled or notched)
Small Stender Dish
Capillary Siphon

Figure 1

The amount of fluid delivered each time the surface film is broken, hence also the total number of deliveries, varies with the diameter of the Stender dish and with the caliber of the delivery tube. If a small number of deliveries, each of large volume, are desired, select a large tube and a wide Stender dish. More deliveries, each of smaller volume, are made when tube and dish are small. The volume of each delivery and the time between deliveries are small at the beginning of the process and increase markedly as the reservoir empties. Other factors influencing somewhat the volume delivered are the shape of the distal end of the delivery tube and the position of this tube.

The duration of performance varies with the rate of delivery of the siphon and the capacity of the reservoir. The duration can be varied over a range permitting emptying of the reservoir in any time from a few minutes to several weeks by selecting the proper ratio of caliber of siphon to capacity of reservoir. The following table gives the approximate duration of performance with water for a 2-liter flask, a 13.5-mm. delivery tube with an 8° bevel, vertically placed, and a 50-mm. Stender dish, when drained by siphons of calibers falling in the most useful range. With such a set-up, about 200 deliveries averaging about 10 cc. per delivery are made.

Number of drops per minute delivered by siphon	*Duration of performance*
50	1 day
32	2 days
25	3 days
20	4 days
18	5 days
16	6 days
15	1 week
10	2 weeks

THE ORIGIN OF PSEUDOTHROMBOCYTES FROM LEUKOCYTES

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TWO FIGURES

Since the first recorded observation of blood platelets there has been considerable discussion concerning their origin. At least five distinct theories have been propounded: 1) that they are the extruded nuclei of the red cells; 2) that they are merely cellular débris; 3) that they are cytoplasmic bits budded from white cells; 4) that the blood platelets are not biological entities, but are only artifacts such as precipitated stain; and, 5) that they are non-nucleated cytoplasmic fragments budded off from the pseudopodia of the megakaryocytes of the bone marrow (Wright, '10). It is the last theory, substantiated by Ogoto ('12), Downey ('13), and Selliger ('23), that has now become the classic theory of the origin of thrombocytes in mammals. While accepting this without reservation, the writer wishes to call attention to a supplementary way in which thrombocytic elements may be formed in man.

In recent years considerable importance has been attached to the thrombocyte count in diseases of the blood. Following the work of Duke and others, it has now become recognized that when the platelet count falls below 100,000 the bleeding time is usually prolonged—an observation that is of particular value in purpura hemorrhagica. However, there are two notable exceptions to this rule—namely, hemophilia and chronic myelogenous leukemia—diseases in which bleeding occurs, notwithstanding the presence of normal or even increased number of platelets.

While studying some twenty-five cases of leukemia during the last five years, chiefly at the Research and Educational Hospital of the University of Illinois, my attention was attracted to the great variation in the number of platelets in the different forms of this disease. That this could not be due to the method of counting was definitely ruled out by extensive experimentation, in which many of the fifty-one methods found in the literature were tried. Finally, all but three methods were eliminated. Employing these three, each of which is based on a different principle, I obtained the highest counts with Thomsen's method, the next with that of Wright and Kinnicutt, and the lowest with Ottenberg's. But in no cases were these variations great enough to be of clinical significance.

It became apparent, therefore, that the great fluctuation in the number of blood platelets in various types of leukemia was not due to the method of counting, but was characteristic of the disease. Thus it was observed that the platelet count was usually less than normal in the small round-cell lymphatic type, in acute leukemia, in subleukemic and aleukemic phases of all types of leukemia, and greater than normal in chronic splenomyelogeneous leukemia. It is this last condition with which this article is primarily concerned.

In each instance the routine blood examination consisted of a study of fresh unstained blood, prepared by placing a drop of blood on a cover-slip, inverting it on a slide, and sealing with vaselin. A stage incubator was usually employed in order to keep the blood at body temperature. In these preparations each specimen was examined for cellular morphology, for approximate white-cell count and number of eosinophiles and activity of the white cells, for the number of platelets, and the time of formation, and for the character and the amount of fibrin formed. It was while studying this last process that a significant feature of chronic splenomyelogenous leukemia was observed. When the fibrin formed, some of the platelets served as a nidus for radiating fibrin (as is seen in normal blood), while other platelets seemed to take no part in the formation of the fibrin network, but just floated about between the fibrin meshes. This pointed to a physiological differentiation of the thrombocytes, but on close and continued observation a genetic difference was noted: for some platelets were actually seen to bud off from amoeboid myelocytes. This actual budding of the cytoplasm was the most important observation, for here under our very eyes were being formed non-nucleated cytoplasmic bodies (plastids) that were indistinguishable from blood platelets (fig. 1). This was then probably the origin of a second type of platelets. This budding was observed many times. Some blood was more active in this respects than others.

These cytoplasmic buds, unless they originated from eosinophilic or basophilic leukocytes, were indistinguishable from true platelets in the counting chamber or fresh preparations of blood. Indeed, it was well nigh impossible to differentiate them in fixed and stained preparations, as the true platelets in leukemia are atypical. The clear peripheral zone which is usually present in normal platelets is often missing in leukemia and therefore cannot be used as a differentiating feature.

When the fixed and stained slide and cover-slip preparations were studied, confirmatory evidence that platelets are formed from myelocytes was afforded by the presence of cytoplasmic bits containing eosinophilic or basophilic granulation, undoubtedly budded from the eosinophilic and basophilic myelocytes. Still other small bits were found in which the cytoplasm resembled that of the polymorphonuclear neutrophiles, lymphocytes, and neutrophilic myelocytes. These various cells were also seen in the actual stages of budding. The old cells showed a much greater tendency to bud than the young cells. Myeloblasts and young myelocytes budded less frequently than older myelocytes.

Another feature which may or may not be significant was the great range of variation in the amount of cytoplasm about the nuclei of the myelocytes, which varied from a wide band to a very narrow rim. This loss of cytoplasm may have been due to cytoplasmic budding (fig. 2). Also, before budding occurred, a heaping of the cytoplasm at the poles was often noticed, such as occurs so frequently in the budding of true platelets from the megakaryocytes (compare cell 8, fig. 2).

For the above reasons it is concluded that the increased platelet count in chronic splenomyelogenous leukemia is due in part at least to the presence of pseudoplatelets budded

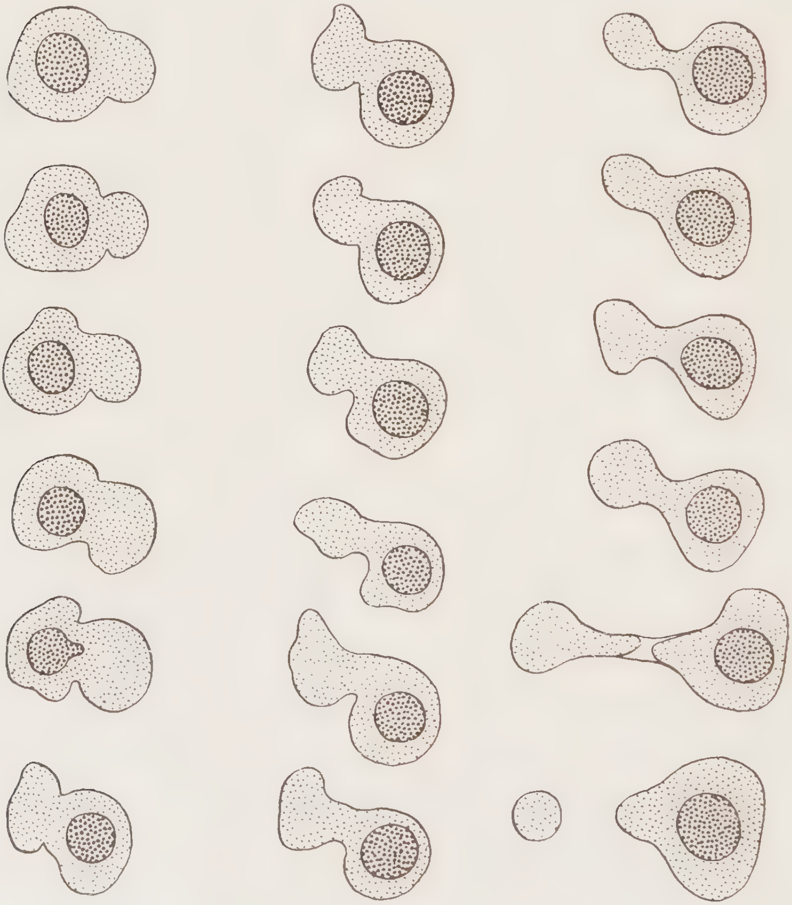

Fig. 1 Camera-lucida sketches of actual budding of cytoplasm from a neutrophilic myelocyte in fresh unstained blood preparation from a case of chronic myelogenous leukemia. Time, two hours and five minutes.

from the leukocytes. These pseudoplatelets take no part in fibrin formation; in fact, they seem quite functionless. This may explain the apparently contradictory observation that an increased platelet count in this disease is accompanied by a great tendency to bleed. Thus, recently Minot and Buckman refer to a case of chronic myelogenous leukemia with a platelet count of 1,500,000 per cubic millimeter in which hemorrhage was a prominent symptom.

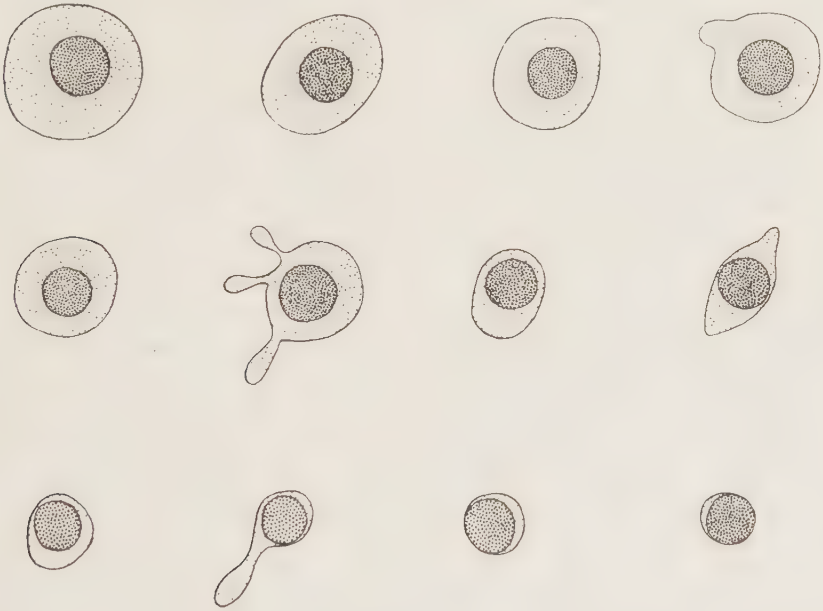

Fig. 2 Camera-lucida sketches of neutrophilic myelocytes from a fixed and stained blood preparation from a case of chronic myelogenous leukemia. Note that the nuclei are approximately the same size, while the cytoplasmic rim varies from a very wide rim represented by the cell in the upper left to the cell in the lower right which shows only a tiny crescentic bit of cytoplasm. Some of the cells show cytoplasmic budding. The cell in the middle row (extreme right) shows polar arrangement of the cytoplasm, which is a common formation prior to budding.

It is also pertinent to note that in a single case of lymphatic leukemia of the large-cell type (the only case which came under my observation) cytoplasmic budding of the large lymphocytes occurred, although to a lesser extent than in myelogenous leukemia.

In 1925, Dr. V. E. Emmel, of this institution, reported this phenomenon in tailed amphibians. He found that it existed consistently and believed it to be a normal occurrence in these lower forms. It remains for further observation to establish whether cytoplasmic budding of the marrow cells is a normal attribute of higher vertebrates, or whether it is merely a latent characteristic developed under the physiological conditions of this disease.

At least the possibility exists that this process of cytoplasmic budding may occur in normal blood. Let us take, for example, a normal blood picture which shows 7000 white blood cells and 300,000 platelets. If each leukocyte were to produce one cytoplasmic bud or pseudoplatelet, the platelet count would be increased by only 7000, making a total count of 307,000, which is well within the limits of experimental error and of no clinical significance. But if we consider a case of chronic myelogenous leukemia with, let us assume, a leukocyte count of 350,000 and a true platelet count of 300,000, then if each of the leukocytes produces one cytoplasmic bud or pseudoplatelet, the total platelet count would be 650,000. Or if each white cell were assumed to give off two cytoplasmic buds, the total count would be more than tripled. Or, finally, if in either of these instances the true platelet count were reduced below 100,000, or below the level where hemorrhage usually occurs, the presence of the pseudoplatelets would still raise the total count above the normal level.

Having decided that there were two types of platelets in chronic myeloid leukemia, an attempt was then made to separate them, in order to determine what proportion of each type existed. Among other methods the effect of hypotonic salt solution was tried as follows:

Blood was drawn by venepuncture, oxalated, and allowed to sediment slowly, so that the red and white cells precipitated, while the platelets remained in a homogeneous suspension in the serum. This serum was carefully transferred to Williamson centrifuge tubes and centrifuged at high speed to separate the platelets from the serum. The supernatant fluid

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was then discarded and the platelets suspended in a little physiological salt solution. The usual set of twelve tubes was set up for a fragility test (from 0.28 per cent to 0.5 per cent sodium-chloride solutions) and a drop of the platelet suspension added to each tube. After about fifteen minutes, a drop from each of these tubes was placed on a slide covered with a cover-slip, sealed with vaselin, and examined with oil-immersion lens. The following observations were made:

There were two definite levels of hemolysis. One was at about 0.42 per cent, where some of the platelets ruptured, lost their granules, and disappeared. The other level was difficult to determine because it was so indefinite, for the remainder of the platelets imbibed a large amount of fluid, increased in size chiefly by elongation, and adhered to one another in large translucent, viscous-looking masses. These two distinct reactions to hypotonic salt solution tended to confirm our belief that we probably had two kinds of platelets. Actual counts of these two types were attempted by making a total count in normal saline of the same dilution and then making another count of tube 5 (0.36 per cent) after the disappearance of the first type of platelets. But the latter count was impossible because of the cohesiveness of the second type of platelets.

The action of hypotonic salt solution on the blood platelets was also observed directly under the microscope by allowing various degrees of hypotonic solutions to flow under the cover-slip and mingle with fresh unstained blood. The same conditions were observed as described in the previous paragraph.

Confirmatory evidence was obtained by a simple comparison of the white-cell counts and the platelet counts in these cases.

1. In no instance was there an increase in the number of blood platelets when the white-cell count was normal or below.
2. The blood-platelet count was not increased and was usually decreased in cases with a high percentage of very young cells.

3. The highest platelet counts were obtained in those cases with a high white count and a small percentage of very young cells.

4. No actual mathematical relationship existed between the two counts (white-cell count and platelet count).

5. The bleeding phenomenon bore no relationship to the platelet count. There were five cases that had a persistently high platelet count (700,000 to 900,000) in which bleeding was a prominent symptom.

The accepted name for these small non-nucleated cytoplasmic bodies is plastids, of which there are several varieties in the blood stream. They may be classified as follows:

- Plastids
  - Erythroplastids
  - Leukoplastids
    - Megakaryocytic plastids (Thrombocytes, true platelets)
    - Leukocytic plastids (Pseudoplatelets)
      - Granular
        - Neutrophilic
          - Polymorphonuclear neutrophiles
          - Myelocytes (neutrophilic)
        - Eosinophilic
          - Eosinophiles
          - Myelocytes (eosinophilic)
        - Basophilic
          - Basophiles
          - Myelocytes (basophilic)
      - Non-granular
        - Lymphocytes
        - Myeloblasts
        - Lymphoblasts

SUMMARY

1. The blood-platelet count in chronic myelogenous leukemia is usually increased, while in small round-cell lymphatic leukemia, acute leukemia of all types, and in subleukemic and aleukemic phases of all types of leukemia the platelet count is usually decreased.

2. This increase in the platelet count in chronic myelogenous leukemia is due, at least in part, to the presence of pseudoplatelets budded from the leukocytes.

3. The pseudoplatelets seem to take no part in fibrin formation.

4. Excessive hemorrhages in cases of chronic splenomyelogenous leukemia with high platelet counts may be explained by a decrease in true platelets below the hemorrhage level of 100,000 and an increase of the pseudoplatelets.

5. The possibility exists that leukoplastids may be normal constituents of the human blood.

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THE EFFECT OF UNDERNOURISHMENT ON THE PROLIFERATIVE ACTIVITY AND STRUCTURE OF THE EPIDERMIS OF THE GUINEA-PIG EAR

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In a preceding paper, Loeb and Haven(1) determined the cell proliferation and the number of cells in the different layers of various parts of the skin epidermis in the normal male guinea-pig; they compared also the variations found at different seasons of the year and at different ages of the animals, and furthermore, they compared the epidermis of rat, pigeon, and chicken with that of the guinea-pig. In a second paper(2) these authors determined the effect of the sexual cycle, of pregnancy, of castration, and of other conditions in the generative organs on the activity and structure of the skin of the guinea-pig. It was shown that the proliferative activity was lowered during the greater part of the sexual cycle and also during castration, but that it rose again during pregnancy, especially during the later stage.

At the time that we studied the structure and proliferation of the skin in the normal guinea-pig, we also carried out some experiments on the effect on the skin of underfed guinea-pigs. The results of these underfeeding experiments are dealt with in this paper.

The figures for the normal guinea-pigs of the first communication serve as controls for these underfed animals. We shall designate as series A these experiments in underfeeding, the data of which contain also the old figures obtained from the normal animals of the previously published report(1).

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In addition, we carried out a new series of underfeeding experiments together with new controls. It was necessary to have new controls with the recent underfeeding experiments, since the time of year, the age, and the state of nourishment of the animal give rise to certain variations in normal counts, as has already been mentioned in our previous papers.

The new control experiments, heretofore unpublished, together with the new underfeeding experiments we shall designate as series B.

Thus, in both series, A and B, the results are controlled by counts in animals examined at the same period of time; furthermore, the counts in normal and underfed animals were made in each case by the same person.

NORMAL GUINEA-PIGS

We shall first report on our findings in normal guinea-pigs used as controls in series B and compare these results with those obtained in series A, which were published in our first paper.

The former counts are seen in tables 1, 2, 3 and 4.

TABLE 1

Number guinea-pigs I[1]	*Number mitoses per 1 mm. skin* II	*Number mitoses per 1000 cells* III	*Number cells in lower layer* IV	*Number cells in upper layer* V
67	2.29	4.81	159	309

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[1] We shall from now on use, for the sake of convenience, only the Roman numerals in order to indicate the meaning of the figures in our tables.

The figures in table 1 represent the averages of individual cases, in which there were considerable deviations from the mean. It is clear that in smaller groups the averages could differ, to a certain extent, from the total averages; thus, in one smaller group which was used for the comparison of the counts in the right and left ears of the same animals, the figures were as seen in table 2.

TABLE 2

I	*II*	*III*	*IV*	*V*
19	1.20	2.65	161	287

Furthermore, during the summer months, the counts of mitoses, as well as of cells in the upper layers, were found to be lower than during the remainder of the year.

In young guinea-pigs, within the first two weeks of life, the counts were lower than in the adult pigs (table 3).

TABLE 3

I	*II*	*III*	*IV*	*V*
27	1.60	3.94	158	252

In table 4 will be seen the variations found with increasing age.

TABLE 4

Variations with increasing age

WEIGHT	I	II	RANGE OF VARIATION IN II	I	V	RANGE OF VARIATION IN V
Slightly below 400 grams (average weight, 382 grams)	18	3.34	0.38–8.37	18	314	254–413
Below 450 grams	24	2.87	0.38–8.37	25	312	254–413
450 to 550 grams	16	2.07	0.64–5.32	17	312	256–360
450 to 600 grams	22	2.07	0.64–5.32	22	314	256–373
Above 550 grams	17	1.61	0.38–3.65	17	302	257–373
Above 600 grams	12	1.35	0.38–1.98	12	294	253–361

In the following tables, 5, 6, 7, 8, and 9, the results of the recent counts on normal guinea-pigs of series B are tabulated. In table 5 it will be noted that three classes of results are given, namely, those of earlier counts, later counts, and counts of very heavy guinea-pigs.

TABLE 5

New counts, series B

Average weights	*I*	*II*	*III*	*IV*	*V*
Earlier counts series B, 393 grams	11	1.80	4.11	150	288
		(Var., 0.90 to 2.84)			(Var., 259 to 325)
Later counts series B, 381 grams	10	2.087	4.97	148	271
		(Var., 1.25 to 3.66)			(Var., 256 to 301)
Heavy guinea-pigs, 736 grams	11	1.23	2.82	152	288
		(Var., 0.14 to 2.08)			(Var., 242 to 342)

The averages of these three classes in table 5, combined, are given in table 6.

TABLE 6

I	*II*	*III*	*IV*	*V*
32	1.69	3.93	1.50	283

If we exclude the heavy guinea-pigs (those weighing over 600 grams), the averages of the remaining animals are as seen in table 7.

TABLE 7

Average weight	*I*	*II*	*III*	*IV*	*V*
387 grams	21	1.94	4.52	149	280

For very young guinea-pigs, that is, five, six, eight, and twelve days old, with a range of weights from 78 to 175 grams, we obtained the figures shown in table 8.

TABLE 8

I	*II*	*III*	*IV*	*V*
7	1.27 (Var., 0.83 to 1.66)	3.13	158	254 (Var., 216 to 336)

A comparison of the results from guinea-pigs, five and six days old, with those of guinea-pigs, eight and twelve days old, shows the figures for the latter to be higher (tables 9a and 9b).

TABLE 9a

Guinea-pigs five and six days old

I	*II*	*III*	*V*
4	1.11	2.8	244

TABLE 9b

Guinea-pigs eight and twelve days old

I	*II*	*III*	*V*
3	1.48	3.58	268

If we compare these results with those shown in the first paper of this series of publications, we find the averages, both for the number of mitoses and for the number of cells in the upper layers, lower in the more recent counts than in the former ones. However, the recent figures come within the range of variations found in the former counts. In the earlier series (A), in general, we find the upper level of range of variations higher than in the later series, and this accounts for the differences in the averages. The fact that, in the first series, there are a number of animals in which the counts are higher than in the second series may be due to variable environmental factors, such as the nourishment of the guinea-pigs, the temperature at which the animals were kept, or to certain other conditions. Notwithstanding these differences in average figures, the relative effect of various factors on these counts is approximately the same in series B as in series A. For example, in both series, in the first week or two of life, the counts are lower than later in life. Again, a decrease takes place in very heavy animals. Inasmuch as no counts in series B were made during the summer months, we can make no comparison between series A and series B as far as the seasonal variations affecting the epidermis are concerned.

EFFECT OF OESTRUS ON THE PROLIFERATION AND STRUCTURE OF THE EPIDERMIS IN THE GUINEA-PIG

In the former investigations, that is, in series A, Loeb and Haven(2) showed that during oestrus the mitotic proliferation of the epidermis was lowered, when compared with that found in normal males. They also examined the effect of oestrus on cell proliferation and structure of epidermis in the recent series B and again found that the proliferative activity is lowered in female guinea-pigs during the period of the sexual cycle. The results of counts made in series B confirming our former conclusion are presented in table 10.

TABLE 10

Average weight	*I*	*II*	*III*	*IV*	*V*
481 grams	6		194	152	319
(Var., 365 to 570 grams)		(Var., 0.28 to 1.45)			(Var., 267 to 440)

In those former experiments it was found that not only was the average cell proliferation lower during oestrus, but that all individual counts were below the average found in normal male guinea-pigs of corresponding weight. The same results were obtained by us in series B, as shown by the following figures. Thus, in series B, the average counts of mitoses per 1 mm. skin in the individual guinea-pigs during oestrus were: 0.55, 0.28, 0.98, 0.83, 1.04, 1.45. In the controls of corresponding weights, the figures were: 1.31, 0.90, 1.18, 2.60, 2.20, 2.84, 2.50, 0.97, 1.10, 1.80, 2.43, 2.29, 1.25, 1.31, 2.50, 2.38, 2.08, 3.66, 2.29, 1.52, 1.59.

The average for cell proliferation, found in this series, in guinea-pigs during oestrus was even lower than the averages obtained in the heavy and very young guinea-pigs. Our present investigations (series B) are less comprehensive than the former ones of Loeb and Haven, but as far as the new studies go, they confirm the former results as to the effect of the cyclic changes in the generative organs on the cell proliferation in the epidermis of the guinea-pig.

EFFECT OF UNDERFEEDING ON THE PROLIFERATION OF THE EPIDERMIS OF THE GUINEA-PIG'S EAR

As will be recalled from above, two experiments were carried out, in which underfed guinea-pigs were used during variable periods of time, one in series A and one in series B.

The animals in both series received the ordinary kinds of food, consisting mainly of grains and green food in ordinary proportions, but given in smaller quantities than usual; only in one guinea-pig was the amount of green vegetables given daily reduced to a minimum, the diet consisting mainly of oats and water. The experiment extended over periods ranging between a few days and about five weeks. The changes in weights in different animals during the period of underfeeding varied considerably, namely, between a loss of about 19 per cent of the original weight and a gain of more than 6 per cent.

In table 11 are seen the averages found in the underfeeding experiment of series A.

If we compare the figures of table 11 with the corresponding ones of the normal controls found in table 1 and table 4, we find the total averages very similar. Although a decrease is seen in the groups with a weight below 400 grams, a slight increase is seen in the group weighing between 400 and 600 grams. The number of cells in the upper layers is slightly higher in the underfed animals.

In fifteen of the nineteen guinea-pigs, we have exact data as to the changes in weight which the animals underwent during the period of underfeeding. In the group with a weight below 400 grams we find the lowest figures for mitoses in those animals which lost the largest amount (15 to 16 per cent) of their initial weight, within a period of nine days or less (table 11). The young guinea-pig, which was fed mainly with oats and water and with a very slight amount of greens during a period of nine days, also showed a much reduced proliferative activity, although the loss in weight was only 5.5 per cent of the initial body weight. In one animal, which lost as little as 3.6 per cent of its weight in ten days, we found only a slight decrease in proliferative activity, while in another animal, which showed a small gain (6.8 per cent) in weight in ten days, we found the mitotic activity to be very high (5.06 mitoses per 1 mm.).

TABLE 11

Averages found in series A

	I	II	III	IV	V
a) Weight below 400 grams. Average weight about 350 grams	7	1.75 (Var., 0.51 to 5.06)	3.63	157	330 (Var., 282 to 409)
b) Weight 400 to 600 grams. Average weight about 432 grams	11	2.95 (Var., 0.76 to 9.23)	5.87	152	323 (Var., 252 to 399)
c) Weight above 600 grams. Average weight 660 grams	1	1.76	3.45	163	347
Total average. Average weight about 405 grams	19	2.44	4.91	154	328

In the next group, those with a weight between 400 and 600 grams, only two guinea-pigs had very low counts of mitoses. These lost 12 to 13 per cent of their body weight within a few days. Two other animals, which had moderately low counts

(1.47 and 1.79) lost 11 to 18 per cent of their weight, one within a few days, the other within a period of twenty-two days. Some animals had normal or higher than normal counts. One of them, which lost 10 per cent of its weight in twenty-two days, had an abnormally high count; also, another that lost as much as 19 per cent in ten days had a count above the average. Furthermore, a few animals that lost less than 10 per cent in five weeks had a count slightly above the average. The last guinea-pig, in this group, gained 4 per cent in weight in ten days and it showed a proliferative activity above the average.

We find, then, in these groups, that the effect of underfeeding on the proliferative activity of the epidermis is greater in the lighter (younger) guinea-pigs than in the heavier (older) ones. Furthermore, we find that there is a certain relationship between the percentage loss in weight and the diminution in proliferative activity, although it can be seen that, with a very marked loss in weight, the mitotic activity may rise above the average.

We shall next consider the more recent underfeeding experiments, namely, those in series B. Tables 12 and 13 (groups A and B) are composed of the results obtained in these investigations.

TABLE 12

Underfeeding

Average weight	*I*	*II*	*III*	*IV*	*V*
377 grams	8	0.94	2.40	158	311
		(Var., 0.41 to 2.10)			(Var., 263 to 365)

It is seen, from table 12, that the average for cell proliferation for underfed guinea-pigs is distinctly below the averages found in the corresponding age classes of normal ones. The number of cells in the upper layers is not decreased; on the contrary, it is somewhat higher in the underfed animals than in the controls. The eight guinea-pigs in table 12 may be separated into two even groups, according to the period of time during which underfeeding took place (group A and group B under table 13).

TABLE 13

Group A—underfed during a period of eleven days—average loss of weight, 27 to 32 per cent

Average weight	*I*	*II*	*III*	*IV*	*V*
385 grams	4	1.26	2.80	156	324
(Var., 365 to 400 grams)		(Var., 0.69 to 2.10)			(Var., 263 to 365)

Group B—underfed during a period of thirty days—average loss of weight, 17 to 19 per cent

Average weight	*I*	*II*	*III*	*IV*	*V*
370 grams	4	0.61	2.01	159	298
(Var., 360 to 395 grams)		(Var., 0.41 to 1.06)			(Var., 283 to 324)

It can be seen that the decrease in proliferative activity is much greater in the group of animals underfed during a period of thirty days than in the group underfed for eleven days, although in the latter the average loss is much greater than in the former. In this series we also find that an individual may lose a great portion of its weight and still show a proliferative activity of the epidermis which is approximately as high as in the normal controls; thus, in one guinea-pig, which lost 32 per cent of its initial body weight within a period of eleven days, we found 2.10 mitoses per 1 mm. skin. The number of upper cells was likewise relatively high in this case, being 336.

Thus, we find that, in series B, the effect of underfeeding is greater than in series A. This may, perhaps, be due to the fact that in this series the average percentage loss in weight is greater than in series A. However, we also cannot exclude the possibility that other factors may enter into the result, because in individual cases there is no strict parallelism between percentage loss in weight and decrease in mitotic activity, as was noted above in the comparison between the guinea-pigs of group A and those of group B. We have also to consider the fact that the average initial weight of the animals was lower in series B than in series A.

CONCLUSIONS

We may conclude that underfeeding tends to decrease the mitotic activity of the ear epidermis of the guinea-pig, but that this change is not accomplished very readily, for it may be resisted, at least, in individual cases, for a considerable period of time.

Furthermore, if we compare the data observed by us, in the epidermis, with the corresponding ones that Gray and Loeb(3), as well as Rabinovitch(4), obtained in the thyroid gland of the guinea-pig, it appears probable that the reduction in proliferative activity through underfeeding can be more readily accomplished in the thyroid gland than in the epidermis and that the latter appears to be relatively stable in its reaction. In contrast with this relative inertness of the epidermis in its reaction to a diminution in the amount of food given is its responsiveness to influences originating in the generative organs during the sexual cycle of the female guinea-pig.

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THE EFFECT OF ADMINISTRATION OF THYROID SUBSTANCE, OF POTASSIUM IODIDE, AND OF EXTIRPATION OF THE GREATER PORTION OF THE THYROID GLAND ON THE EPIDERMIS OF THE GUINEA-PIG

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INTRODUCTION

In a previous investigation(1) we found a very marked effect of the functional states of the female generative organs and of the internal secretions of the ovary on the proliferative activity and structure (number of cells) of the guinea-pig epidermis. We now continue these studies, investigating the effect of another important gland with internal secretions, namely, the thyroid. The influence of a surplus of, as well as of a deficiency in, thyroid hormones on the epidermis was tested. In particular, the effect of a single dose of thyroid substance, and also of daily doses given over definite periods of time, was studied.

In addition, we tested the effect of partial thyroidectomy on the epidermis and then the result of multiple doses of thyroid substance in guinea-pigs in which the greater part of the thyroid gland had previously been extirpated. Lastly, we compared the action of the thyroid hormone with that of a substance intimately connected with the function of the thyroid gland, namely, iodine, in the form of potassium iodide, on the proliferative activity and structure of the epidermis.

One reason for selecting thyroid substance and potassium iodide for these experiments was that in former studies(2, 3) we found that these two substances produced opposite effects on the cell proliferation and structure of the acini of the thyroid gland of the guinea-pig. We also selected the hormone substance of the thyroid gland for this study, because it is known that this gland affects differentiation of tissues, accelerating metamorphosis in the tadpole; furthermore, according to some authors, it promotes the regenerative growth of peripheral nerves and of bone.

A number of years ago, we showed that the internal secretions of the ovary, in association with hereditary factors, exert a definite effect on the development of mammary cancer in mice, and that this effect can be determined in a quantitative manner(4). In our previous publications concerning the influence of ovarian secretion, we suggested that perhaps other internal secretions may play a similar rôle in the development of certain types of cancer similar to that established in the case of the ovary(4). With this thought in mind, we undertook the present quantitative study of the effect of the thyroid gland and its hormone and also of potassium iodide on the growth processes of the epidermis of the guinea-pig ear. As in the preceding paper dealing with the effects of underfeeding, older experiments of series A and more recent ones of series B are distinguished.

THE EFFECT OF REPEATED DOSES OF THYROID SUBSTANCE ON THE CELL PROLIFERATION IN THE EPIDERMIS OF NORMAL MALE GUINEA-PIGS

Series A

One or two tablets, each containing 0.1 gram of thyroid substance (Armour & Co.) and, in several cases, one-half of a tablet, were fed daily. The feeding extended over periods varying between five and forty days. In general, no definite relation between the number of mitoses and length of the period, during which the substance was given, could be demonstrated. However, in two animals, in which the period of feeding was longest (thirty to forty days), the counts were above those found in the averages of normal guinea-pigs. As a rule, the cell number was highest in those animals in which the feeding took place over the longer periods of time.

Table 1 presents the average figures for a group weighing between 400 and 600 grams, treated as described above.

TABLE 1

Average weight	*I*[1]	*II*	*III*	*IV*	*V*
517 grams	16	2.01	4.51	165	286
		(Var., 0.64 to 3.65)			(Var., 218 to 388)

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[1] The Roman figures have the same significance as in the preceding paper.

The average of results obtained with heavier guinea-pigs, those weighing above 600 grams, is shown in table 2. The thyroid feeding extended over periods varying between six and sixty days. Again, no definite relation could be observed between the length of these periods and the mitotic proliferation. For example, in an animal fed for sixty days, the number of mitoses was 1.92 per 1 mm. skin; another, fed the same amount, gave a figure of 1.69 after only six days.

TABLE 2

Average weight	*I*	*II*	*III*	*IV*	*V*
643 grams	8	1.27	2.90	162	254
		(Var., 0.44 to 1.92)			(Var., 259 to 325)

The results of the total average of twenty-four guinea-pigs considered above and of five additional ones which were treated for periods of eighteen and twenty days, but the weights of which were lost, are presented in table 3.

TABLE 3

I	*II*	*III*	*IV*	*V*
29	1.72	3.85	165	285
	(Var., 0.50 to 4.31)			(Var., 196 to 388)

Comparing these data with those of the controls, given in the preceding paper, we may conclude that daily administration of thyroid substance extending over periods varying between five and sixty days does not lead to an increase in proliferative activity of the epidermis in the guinea-pig. The averages of mitoses are actually somewhat lower than those of the controls, but it is doubtful how much significance can be attached to these differences. They may be due to the loss of weight, which a number of animals showed under the influence of thyroid administration, or to some other accidental factors. Only by excluding these possibilities could we attribute to thyroid feeding a specific lowering effect on the proliferative activity of epidermis.

As to the number of cells found in the lower layer of the epidermis, the average was about the same as in the controls, while for the upper layers the average number was slightly below that of the normal animals, but well within the range of variations there observed. Therefore, we are justified in drawing the above conclusion, that prolonged feeding of thyroid substance does not increase the number of cells in the epidermis.

EFFECT OF THYROID FEEDING ON THE EPIDERMIS IN PREVIOUSLY THYROIDECTOMIZED GUINEA-PIGS

Series A

In six animals, two weighing below 400 grams (average, 329 grams), three weighing between 400 and 600 grams (average, 549 grams), and one weighing 660 grams, all but a small part of one lobe of the thyroid gland was extirpated. Following this operation, 0.1 to 0.2 gram thyroid substance was administered orally, over periods ranging between five and twenty days.

The average figures obtained in this group are seen in table 4.

TABLE 4

I	*II*	*III*	*IV*	*V*
6	2.76 (Var., 0.51 to 6.28)	5.55	153	321 (Var., 270 to 408)

It will be seen that a slight increase was found in the number of mitoses and also in the number of cells. At the present time, we do not wish to discuss the significance of this increase, but shall limit ourselves to the conclusion that feeding thyroid substance after an incomplete thyroidectomy does not diminish either the proliferative activity or the number of cells in the epidermis.

THE EFFECT OF A SINGLE DOSE OF THYROID SUBSTANCE ON THE EPIDERMIS OF THE GUINEA-PIG

Series A

One dose, consisting of four tablets of thyroid substance (Armour & Co.) was administered orally to six guinea-pigs, some of which had been starved for two days previous to the feeding, so that absorption of the material ingested would be hastened. The fasting animals weighed slightly below 400 grams. In five guinea-pigs examination took place five to seven hours following feeding of the thyroid substance, and in one animal it occurred twenty-four hours later. The results are shown in table 5.

TABLE 5

Average weight	*I*	*II*	*III*	*IV*	*V*
381 grams	6	1.79	3.63	162	323
		(Var., 1.02 to 3.07)			(Var., 293 to 381)

We see in this group a reduction in the number of mitoses. However, undernourishment may play a part in this result. The number of cells, on the other hand, is normal or slightly above the average.

TABLE 6

Average weight	*I*	*II*	*III*	*IV*	*V*
451 grams	7	2.27	5.04	160	281
		(Var., 0.32 to 4.74)			(Var., 272 to 325)

Table 6 gives the results for the heavier guinea-pigs, those weighing between 400 and 600 grams.

Here, the average number of mitoses is even slightly above the average of the controls. Examination took place in three cases five to seven hours, in three cases ten to twenty-four hours, and in one case forty-eight hours after administration of thyroid substance. In the three guinea-pigs examined after five to seven hours, the average number of mitoses per 1 mm. skin was 2.71; after fifteen to twenty-four hours, it was 2.49, and in one examined after forty-eight hours it was 0.32.

Series B

Seventeen guinea-pigs ranging in weight between 215 and 425 grams were examined. Each animal was fed 0.1 gram thyroid substance (Armour & Co.). Examination took place six, eight, twelve, twenty-four, and forty-eight hours afterward. The results are presented in table 7.

TABLE 7

Average weight	*I*	*II*	*III*	*IV*	*V*
333 grams	17	1.27	2.87	155	287
		(Var., 0.35 to 2.32)			(Var., 262 to 319)

In table 8, which follows, figures showing the variations with the time of examination are tabulated.

TABLE 8

Time of examination, hours	*Average weight, grams*	*Number of guinea-pigs*	*Average number of mitoses per 1 mm. skin*
6	410	2	1.52 (Var., 0.97 to 2.08)
8	396	3	1.61 (Var., 1.11 to 2.00)
12	412	2	1.80 (Both 1.80)
24	288	7	0.76 (Var., 0.35 to 1.10)
48	365	3	1.61 (Var., 1.13 to 2.32)

The average in this group is below that of the control animals of series B, being 1.27 in the thyroid-fed guinea-pigs, as compared with 1.97 in the control animals. The most marked reduction was observed in the animals examined twenty-four hours after feeding thyroid substance; in these guinea-pigs the weight was also lower than in the others. In evaluating the differences in the results of single-dose feeding in the corresponding animals of series A and series B we must take into consideration the fact that in this series (B) the average weight of the animals was somewhat lower than in the corresponding guinea-pigs of series A.

We may conclude that single doses of thyroid substance do not increase the cell proliferation in the epidermis, nor do they noticeably change the number of cells; on the other hand, they may produce a relatively slight diminution in proliferative activity, especially in the animals weighing less than 350 grams.

THE EFFECT OF THYROIDECTOMY ON THE EPIDERMIS OF THE GUINEA-PIG

Series B

In twenty-five guinea-pigs one lobe and three-fourths of the second of the thyroid gland were extirpated. The isthmus connecting the lobes in guinea-pigs was left intact.

Definite compensatory hypertrophy of the remaining portion of the gland began to set in, in many cases, within ten days following the operation. However, in some cases the hypertrophy was very slight or even lacking. In our experiments examination of the epidermis took place at periods varying between six and thirty-two days following thyroidectomy. We found no definite relation between the length of these periods and the counts obtained. The total averages of this group will be found in table 9.

TABLE 9

I	*II*	*III*	*IV*	*V*
25	2.74	5.57	159	331
	(Var., 1.02 to 6.79)			(Var., 230 to 418)

We have a record of the weight in only eleven of these animals.

The averages for the small group weighing less than 400 grams are given in table 10 and for those between 400 and 600 grams, in table 11.

TABLE 10

Average weight	*I*	*II*	*III*	*IV*	*V*
353 grams	3	3.15	6.43	161	366
		(Var., 3.02 to 3.38)			(Var., 331 to 386)

TABLE 11

Average weight	*I*	*II*	*III*	*IV*	*V*
478 grams	8	2.69	5.44	166	337
		(Var., 1.84 to 5.00)			(Var., 286 to 382)
Total, 444 grams	11	2.81	5.71	164	345

We may conclude from these results that in guinea-pigs with the greater part of the thyroid extirpated, the proliferative activity and the number of cells in the epidermis remain normal or possibly become slightly increased. A diminution in either mitoses or cell numbers cannot be demonstrated in this group.

EFFECT OF MULTIPLE DOSES OF POTASSIUM IODIDE ON THE EPIDERMIS OF NORMAL MALE GUINEA-PIGS

Series A

Doses of either 0.01, 0.05, or 0.1 gram potassium iodide were given daily by mouth. There was no definite difference between the effects of these various doses. The periods during which the potassium iodide was administered varied between five and sixty days, and in one case it extended over 108 days. In several animals the proliferative activity was found to be very low, after eight to ten days' feeding with potassium iodide, but in other cases it was still normal after ten days; in some cases it was low even after thirty days. In two guinea-pigs which had received daily doses of potassium iodide over periods of sixty days, the number of mitoses per 1 mm. of skin was 1.85 and 1.43, respectively, figures which were only slightly below the normal average. In the one case, which extended over 108 days in an animal weighing 735 grams, the number of mitoses per 1 mm. skin was 2.24. Thus there was no reduction in the activity in this instance. Also, the number of cells was within the normal range in those animals examined after sixty and 108 days of feeding. The total number of thirty-five guinea-pigs has been divided into three groups according to their weights, namely, those weighing below 400 grams, those between 400 and 600 grams, and those above 600 grams. The average figures for these different groups are given in tables 12, 13, and 14, respectively. Table 15 gives the total averages for the whole series, that is, a combination of the above three groups.

TABLE 12

	I	*II*	*III*	*IV*	*V*
360 grams	4	0.47	2.89	172	273
		(Var., 0.19 to 1.02)			(Var., 259 to 286)

TABLE 13

	I	*II*	*III*	*IV*	*V*
492 grams	20	1.37	2.93	158	304
		(Var., 0.12 to 3.58)			(Var., 225 to 353)

TABLE 14

	I	*II*	*III*	*IV*	*V*
672 grams	11	1.54	3.49	160	282
		(Var., 0.32 to 2.62)			(Var., 230 to 359)

TABLE 15

Average end weight	*I*	*II*	*III*	*IV*	*V*
505 grams	35	1.32	2.89	161	294
		(Var., 0.12 to 3.58)			(Var., 225 to 359)

We may conclude from these results that the daily administration of potassium iodide to guinea-pigs, during certain variable periods of time, does not increase either the proliferative activity of the epidermis or the number of cells of which it is composed. Actually, the proliferative activity in this series was below that of normal guinea-pigs and even slightly below that of guinea-pigs fed with thyroid substance. The number of cells in the upper layers of the epidermis was also slightly below the average found in normal guinea-pigs, but was still well within the range of normal variations. The changes in proliferative activity took place in these guinea-pigs, notwithstanding the gain in weight, which these animals usually showed, during the progress of the experiment.

THE EFFECT OF MULTIPLE DOSES OF POTASSIUM IODIDE ON THE EPIDERMIS OF GUINEA-PIGS, FOLLOWING EXTIRPATION OF THE GREATER PORTION OF THE THYROID GLAND

In a group of male guinea-pigs one lobe and the greater part of the second lobe were extirpated, and subsequently potassium iodide, in daily doses which in different guinea-pigs varied between 0.01 and 0.1 gram, was administered orally, during periods of time varying between four and thirty-two days. No definite relation was found between the epidermal proliferation and the length of the period during which the potassium iodide was administered or the amount of potassium iodide given.

As was recorded in the preceding series, the total number of animals have been similarly divided into three groups, according to their weights.

In table 16 are recorded the average figures for those weighing below 400 grams; in table 17, for those weighing 400 and 600 grams, and table 18, for those above 600 grams. Table 19 gives averages for all the groups combined.

TABLE 16

	I	*II*	*III*	*IV*	*V*
365 grams	1	2.10	4.69	158	288

TABLE 17

	I	*II*	*III*	*IV*	*V*
504 grams	17	2.38	5.63	162	322
		(Var., 1.45 to 4.29)			(Var., 262 to 380)

TABLE 18

	I	*II*	*III*	*IV*	*V*
614 grams	2	1.48	3.12	162	312
		(Var., 1.37 to 1.60)			(Var., 278 to 347)

TABLE 19

Average weights	*I*	*II*	*III*	*IV*	*V*
Total, 508 grams	20	2.27	5.33	162	320
		(Var., 1.37 to 4.29)			(Var., 262 to 380)

As we have seen above, administration of thyroid substance to not quite completely thyroidectomized guinea-pigs is not followed by a lowering of the proliferative activity of the epidermis.

Similarly, we find in this series that after previous removal of the greater part of the thyroid gland, the lowering of epidermal proliferation which we observed in normal guinea-pigs fed either with thyroid substance or with potassium iodide was lacking. In both cases the counts of mitoses became either normal or even slightly above normal in the thyroidectomized animals. We may, therefore, draw the conclusion that in such guinea-pigs a definite deviation from the normal intensity of proliferative activity is not found. As far as the number of cells composing the epidermis is concerned, it is approximately normal, although it is possible that the number of cells in the upper layers is very slightly increased. We find, therefore, complete parallelism in the conditions found in the thyroid-fed and in the potassium-iodide-fed animals, after a preceding incomplete thyroidectomy.

Our counts suggest that thyroidectomy, even if it is not quite complete, prevents the lowering of proliferative activity, which we observed in animals not thyroidectomized, and that the effects of this operation and those of administration of thyroid substance or of potassium iodide, to a certain extent, balance each other as far as the epidermis is concerned. However that may be, we can be certain that the epidermis of the thyroidectomized guinea-pigs fed with thyroid substance or with potassium iodide remains approximately normal.

THE EFFECT OF THE ADMINISTRATION OF SINGLE DOSES OF POTASSIUM IODIDE ON THE EPIDERMIS OF THE GUINEA-PIG

Series A

Four guinea-pigs, the weights of which varied between 360 and 500 grams, were used in this experiment. To three animals 0.1 gram potassium iodide was given orally and to the fourth, twice this amount. In the first three guinea-pigs, which were examined eight to nine hours after giving the potassium iodide, the number of mitoses and of upper cells was found to be diminished. In the animal which received 0.2 gram potassium iodide, the mitoses count after forty-eight hours was almost normal, while the number of cells (348) in the upper layer was above the average. In table 20 will be seen the average figures for the entire group.

TABLE 20

Average weight	*I*	*II*	*III*	*IV*	*V*
442 grams	4	0.98	2.10	158	281
		(Var., 0.80 to 2.11)			(Var., 251 to 348)

Series B

A single dose of 0.1 gram potassium iodide was administered to each of twelve male guinea-pigs, the weight of which ranged between 300 and 410 grams. Examination took place six, eight, twelve, and twenty-four hours following the feeding.

Average weight	*I*	*II*	*III*	*IV*	*V*
370 grams	12	1.23	2.78	157	289
		(Var., 0.62 to 1.94)			(Var., 241 to 319)

In table 21 are tabulated the average results for the guinea-pigs, arranged according to the number of hours which were allowed to elapse between the time of feeding and the time of examination.

TABLE 21

Mitoses and number of cells at different time periods

Period, hours	*Average weight, grams*	*I*	*II*	*V*
6	392	3	1.68	317
			(Var., 1.31 to 1.94)	(Var., 315 to 319)
8	398	3	1.34	291
			(Var., 1.11 to 1.66)	(Var., 249 to 328)
12	370	3	0.94	289
			(Var., 0.62 to 1.52)	(Var., 272 to 311)
24	323	3	1.08	259
			(Var., 0.90 to 1.25)	(Var., 241 to 291)

From the table it will be seen that the decrease in number of mitoses is most marked after twelve and twenty-four hours. If potassium iodide should actually diminish the proliferative activity in guinea-pigs, it might reasonably be expected that the effect would become noticeable between eight and twenty-four hours, and this appeared to be the case in both series A and B. The time values in this series were similar to those obtained after feeding a single dose of thyroid substance, only in the latter the effect appeared somewhat more slowly, which might be expected, considering the difference in the character of potassium iodide and thyroid. However, our results only suggest such a conclusion; a larger number of experiments would be necessary to establish this time relation definitely. At present, we may state that, in our experiments, a single dose of potassium iodide administered to fairly young guinea-pigs diminished the number of mitoses within twenty-four hours; the number of cells remained about the same as in the controls of series B.

DISCUSSION

In evaluating our results we can consider them, as we have to do in the case of all statistical data, merely as more or less close approximations. As far as the effect of administration of thyroid substance is concerned, we may be certain that it neither increases the proliferative activity of the epidermis nor the number of epidermal cells. This is true of the administration of multiple doses extending over various periods of time, as well as of single doses. On the other hand, it is possible that it leads, in both cases, to a slight, or, occasionally, even to a more marked, lowering of proliferative activity and perhaps of the number of epidermal cells as well. After single doses such a lowering effect was observed, especially twenty-four hours after administration of thyroid substance. However, complicating factors, such as the loss of weight, effected by thyroid feeding, and the age of the animals, are to be dealt with. The youngest were the most affected. We must be content, at present, to state that our results prove that the feeding of thyroid substance to normal guinea-pigs does not increase the proliferative activity of the epidermis nor the number of cells in the epidermis; on the other hand, they suggest a depressing effect due to this substance.

Such a depressing effect, however, was absent when multiple doses of thyroid substance were administered to animals in which the greater part of the thyroid had previously been removed. Under these conditions there was even a suggestion of a slight rise in proliferative activity and in the number of cells in the epidermis.

As we should expect from the results obtained by us in thyroidectomized guinea-pigs in which we administered at the same time thyroid substance, we found that thyroidectomy, even when not complete, does not lower the proliferative activity or the number of cells in the epidermis, but on the contrary, may raise both in experiments in which thyroid substance has not been fed.

As far as the relation of the thyroid gland to the epidermis is concerned, we have obtained no indication that this gland may function as an agent in increasing the proliferating power of the epidermis; and there is therefore no probability that it may play a part in initiating carcinomatous proliferation of the skin or in aiding it, in the sense in which we found that the ovary, through its internal secretion, influences the development of carcinoma of the mammary gland.

How far these findings are applicable to other species we cannot be certain at the present time. In human and animal cretinism, secondary changes, in the connective-tissue constituent of the skin, enter as complicating factors, so that the results which we obtained in the guinea-pig and conditions as cretinism in man and in certain other mammals are not directly comparable.

As to the effects of administration of potassium iodide on the epidermis, we find a certain parallelism between its action and that of thyroid substance.

Multiple doses of potassium iodide, as of thyroid substance, administered by mouth do not increase cell proliferation in the epidermis of the guinea-pig, neither do they cause a rise in the number of epidermal cells. On the contrary, we found a lowering of the proliferative activity and possibly also the number of cells in the epidermis. Furthermore, there is no doubt that the effect produced by administration of potassium iodide cannot be attributed to a loss in weight of the animals during the experiment.

As in the case of administration of thyroid, so also in the case of feeding of potassium iodide, a previous removal of the greater part of the thyroid gland prevented the lowering in epidermal proliferation otherwise effected by the administration of the latter substance.

Furthermore, as after administration of single doses of thyroid substance, similarly, single doses of potassium iodide did not increase the proliferation or the number of cells in the epidermis, but on the contrary, decreased both. Therefore, again there is a very strong suggestion that not only in our experiments, but as a general rule, the potassium iodide in single doses may lower the proliferation and possibly also the number of epidermal cells. This effect seems to be pronounced especially twelve to twenty-four hours after administration of this substance. However, as stated above, we consider the latter conclusion, at present, as merely tentative. On the other hand, we can be certain that single doses of potassium iodide do not raise the epidermal proliferation within the ranges of time used in our experiments.

We see, then, that in contrast to the effects of potassium iodide on the thyroid gland of the guinea-pig, where this substance increases the proliferative activity of the acinus cells in a remarkable manner, it has no such effect on the epidermis, and, on the contrary, it may possibly even lower the multiplication of cells in the latter tissue. Whereas administration of thyroid substance and potassium iodide have opposite effects on the thyroid gland, these two substances have similar effects on the epidermis. This is presumably due to the fact that potassium iodide exerts a direct stimulating effect on the thyroid gland and possibly leads to an increased production of the hormone, whereas administration of thyroid substance removes the incentive to activity on the part of the gland. In the case of the epidermis, the effect of potassium iodide may be an indirect one, perhaps due to excess production and mobilization of thyroid hormone. In tissues other than the thyroid gland, potassium iodide does not, therefore, need to exert a stimulating effect; on the contrary, there is a suggestion that it may lower their activity, as we have seen in the case of the epidermis of the guinea-pig.

SUMMARY

Administration of thyroid substance as well as of potassium iodide to guinea-pigs does not lead to a rise in proliferative activity of the epidermis or to an increase in the number of epidermal cells. On the contrary, there is a suggestion that both multiple as well as single doses of these two substances may cause a lowering in both proliferative activity and number of cells.

After a not quite complete thyroidectomy, no lowering of the proliferative activity in the epidermis nor of the number of epidermal cells in the guinea-pig takes place; there is even a suggestion that it may increase the number of cells. In accordance with these findings, we observed that after a previous (incomplete) thyroidectomy, administration of thyroid substance and of potassium iodide did not lead to a lowering of cell proliferation or of the number of epidermal cells.

The effects of these substances on the epidermis are compared with their effects on the thyroid gland, and furthermore the bearing of our findings on the problem of the significance of internal secretions in the origin of cancer is discussed.

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THE CHANGES IN THE MESONEPHRIC TUBULES OF HUMAN EMBRYOS TEN TO TWELVE WEEKS OLD

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THREE FIGURES

In 1912, Felix, in his treatise "The Development of the Urogenital Organs," stated that the mesonephric tubules in man begin degenerating at 7 mm. and that by the time the 22-mm. stage is reached none is intact. This was generally accepted until 1916, when Bremer expressed the opinion that sound mesonephric tubules may persist as late as the 37- or 40-mm. stage. His conclusions were based on the observation that about a dozen apparently healthy glomeruli are present in embryos of that size. He was able, moreover, to trace intact tubules from some of these glomeruli to the wolffian duct. Other observers, including Lewis and Shikinami, have since called attention to the presence of numerous healthy tubules in the mesonephroi of embryos of about 23 mm., and both von Winiwarter and Wilson have noted what looked like healthy mesonephric tissue as late as the 35- or 40-mm. stage, but the testimony of the two latter, based merely on histological evidence, must be considered inconclusive, as will be seen later.

In an attempt to throw additional light on the subject, it was decided to study carefully the number, disposition, and condition of the tubules in the mesonephroi of human embryos at various stages from about 30 to 40 mm.

The material used in this investigation consisted of sections of the following embryos:

No.	*Sex*	*Length, crown–rump*	*Sections*	*Stain*
H.E.C. 2043	♀	31 mm.	10 μ transverse	Alum cochineal and orange G
H.E.C. 2050	♂	36 mm.	10 μ transverse	Alum cochineal and orange G
H.E.C. 1917	♀	40 mm.	12 μ frontal	Alum cochineal and orange G
H.E.C. 838	♂	42 mm.	14 μ transverse	Borax carmine and Lyons blue

Camera-lucida drawings were made from these serial sections at a magnification of 150 diameters and were used in making graphic reconstructions of the mesonephroi studied.

OBSERVATIONS

Microscopic examination shows the wolffian body to be rather sharply divided into two regions, an anterior, which later gives rise to the vasa efferentia and ductus epididymidis or to the epoophoron, and a posterior, in which the tubules ultimately either disappear or contribute to the paradidymis or paroophoron.

The changes that occur in the tubules of the anterior portions of the mesonephroi of embryos 31 to 42 mm. long have been adequately described by von Winiwarter and by Wilson and need not be gone into at any great length here. They consist of a gradual dedifferentiation, beginning anteriorly and proceeding caudally to about the middle of the wolffian body. The picture is essentially the same in all of the embryos studied, except that the older embryos have proportionately more tubules in the last stages of the dedifferentiation.

Lewis has described the mature human mesonephric tubule as consisting of a capsule, a thick secretory tubule, and a thinner collecting tubule. On microscopic examination, the glomerulus is seen to be lined with a flat epithelium and to contain a capillary tuft full of blood corpuscles. Afferent and efferent arterioles can be seen. The secretory tubule is lined with cells, each of which contains a round or oval nucleus surrounded by a fairly large amount of granular cytoplasm. The nucleus may be situated either centrally or basally. Cell boundaries are indistinct, except, of course, where they impinge upon the lumen. Here, though well defined, they may be quite irregular. This, coupled with the fact that the cells differ considerably in height, tends to make the width of the lumen vary appreciably from point to point.

The junction of the secretory and collecting tubules is fairly well marked, as the appearance of the former is quite different from that of the latter. The epithelium of the collecting tubule consists of small cuboid cells containing round centrally placed nuclei in cytoplasm that is denser, though less granular, than that of the cells of the secretory tubule. Cell boundaries are well defined and the lumen is quite regular. The entire mesonephric tubule, from capsule to wolffian duct, is separated from the surrounding connective tissue by a fine continuous basement membrane.

The first stage in the process of dedifferentiation observed in the anterior halves of the wolffian bodies studied is a thickening of the epithelium of the capsule. A little later, the number of capillaries in the glomerular tuft becomes smaller. Simultaneously the cells in the secretory tubule begin to decrease in size and stain like the epithelium of the connecting tubule. The lumen of the tubule is seen to contain much débris. Reconstruction of the tubules undergoing these changes shows them to be diminishing in length, although the continuity from duct to capsule is still maintained (figs. 1 and 2). The presence of débris in the lumen must be due to the loss of cells from the tubule, since the marked decrease in the length of the tubule can be accounted for only to a slight extent by the shrinkage of the individual cells. A considerable number of them must drop out before the tubule can become as short as it does.

This process continues. The tubule becomes thinner and much shorter and loses some of its curves; the cells of the tubular epithelium become more uniform in size and staining reaction; the capsular epithelium becomes more definitely cuboid and columnar; and the tuft becomes increasingly dense as its cells thicken and its capillaries withdraw. The remains of the glomerulus are finally extruded, the capsule peeling itself off the tuft to become a spherical vesicle enclosed by a single layer of cells now indistinguishable from those of the rest of the tubule.

The gonadic tissue has in the meantime been encroaching on the wolffian body so that now a number of the dedifferentiated capsules are surrounded by cells derived from the testis or ovary.

At about this time a few of the changing tubules lose their continuity, becoming broken either at the junction of the tubule

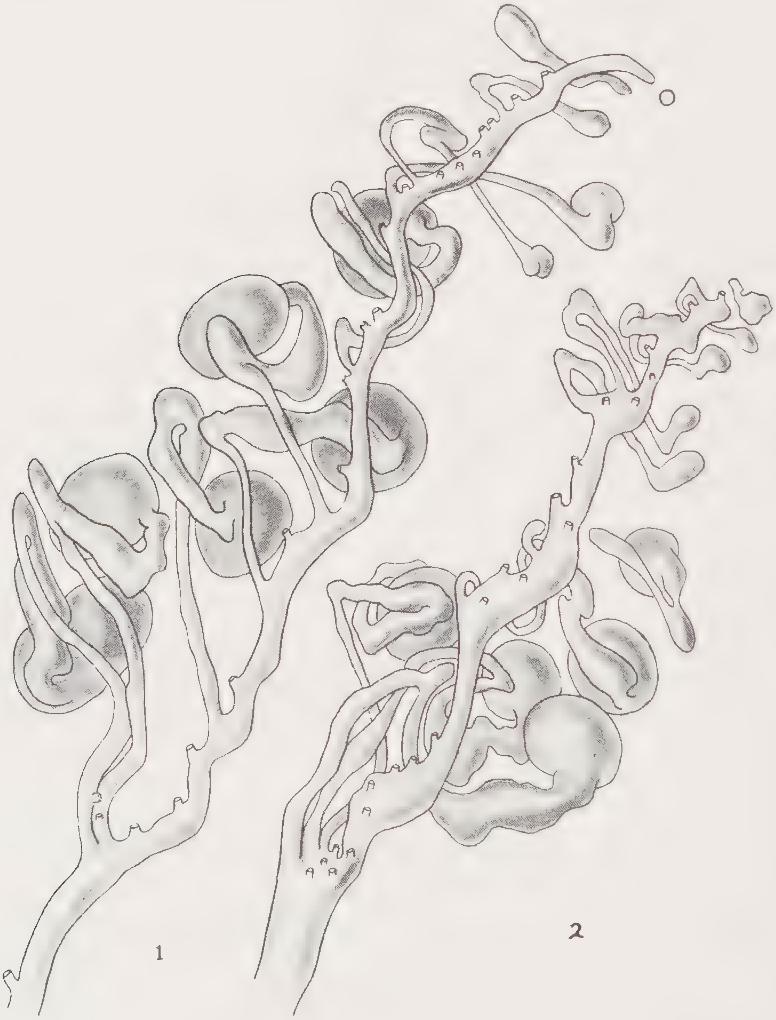

Fig. 1 Reconstruction of part of the right mesonephros of a human embryo of 36 mm.

Fig. 2 Reconstruction of part of the right mesonephros of a human embryo of 31 mm.

and the mesonephric duct or of the tubule and the capsule. This usually occurs in two or three of the most anteriorly situated tubules and less frequently in the other portions of what is to become the epididymis or epoophoron. These broken tubules will, of course, contribute to the paradidymis or paroophoron, while some or all of the dozen or so tubules that still maintain their continuity will give rise to the vasa efferentia of the epididymis.

The extreme anterior tip of the wolffian duct may degenerate, thus cutting off a tubule or two. A tubule isolated in this way, or by degeneration of its own tissue at its junction with the wolffian duct, may swell up and become vesicular. This has been observed only at the anterior end of the organ and is interpreted as being an early stage in the development of a hydatid of Morgagni.

This account of the metamorphosis of the cephalic portion of the wolffian body in human embryos is entirely in harmony with that of von Winiwarter and differs only slightly from that of Wilson.

The process going on in the posterior halves of the mesonephroi of the embryos studied differs entirely from that just described. On microscopic examination the caudal portions of embryos of 31 to 42 mm. seem to consist largely of healthy tubules and glomeruli embedded in a stroma of young connective tissue. There are numerous capsules containing blood-laden capillary tufts, most of which may be seen to join normal afferent or efferent arterioles. Scattered about in the sections are segments of secretory and collecting tubules, each lined with its characteristic epithelium.

If reconstructions of this region are made, it is found that a number of the apparently sound tubules are not continuous from wolffian duct to glomerulus, but are broken in one or more places. Occasionally there is no actual discontinuity, the tubule being reduced to a thin lumenless cord of cells.

It is not possible to distinguish between the intact and most of the broken tubules histologically. This and the fact that the fragments of the broken tubules often maintain their relative positions make it appear that there are more sound ones than there really are. Thus embryos of 40 and 42 mm. were at first thought to contain about a dozen intact tubules, but careful reconstruction showed that there was none.

Undoubtedly the discrepancies in the published statements as to the final time of degeneration of all tubules are due to the persistence of the normal appearance of glomeruli and tubules after actual continuity has been lost.

At 36 mm. it is possible to demonstrate ten continuous tubules. Figure 1 is a reconstruction of the right mesonephros of a male embryo of that size and shows five of these.

It is seen that at this stage the wolffian body has a wavy duct running from a dorsolateral position in the cephalic portion of the organ to a ventrolateral position more posteriorly. The thirty-one tubules spring from it at irregular intervals, generally from its dorsal or medial aspect. The most posteriorly situated tubules run forward from their points of origin and those of the anterior portion run caudally, thus tending to make the mesonephros more compact. The tubules are not arranged linearly, the various parts of one being overlapped by portions of several others when viewed from the side. The healthy tubules, which are located posteriorly, are seen to be of exactly the same shape and arrangement as those described by Lewis in embryos of 23 mm.

Only ten of the eighteen tubules in the posterior portion of this mesonephros are found to be intact, but a larger number can be demonstrated in younger embryos. The organ pictured in figure 2 is that of a female embryo of 31 mm. Among its twenty caudal tubules are fifteen sound ones, of which four are shown. Its duct is less wavy than that of the 36-mm. embryo, it has six more tubules, and the entire organ is shorter and hence more compact, but its general arrangement is the same. In the stages studied there is no difference between the sexes in regard to the wolffian body, although the gonad already shows considerable differentiation.

The point at which the tubules become discontinuous varies. Breaks have been noted at the junction of the glomerulus and secretory tubule, in the secretory tubule, at or immediately below the junction of the secretory and collecting tubules (the most frequent site), and in the collecting tubule itself. Some tubules are broken at more than one point. A number of these discontinuous tubules are shown in figure 3.

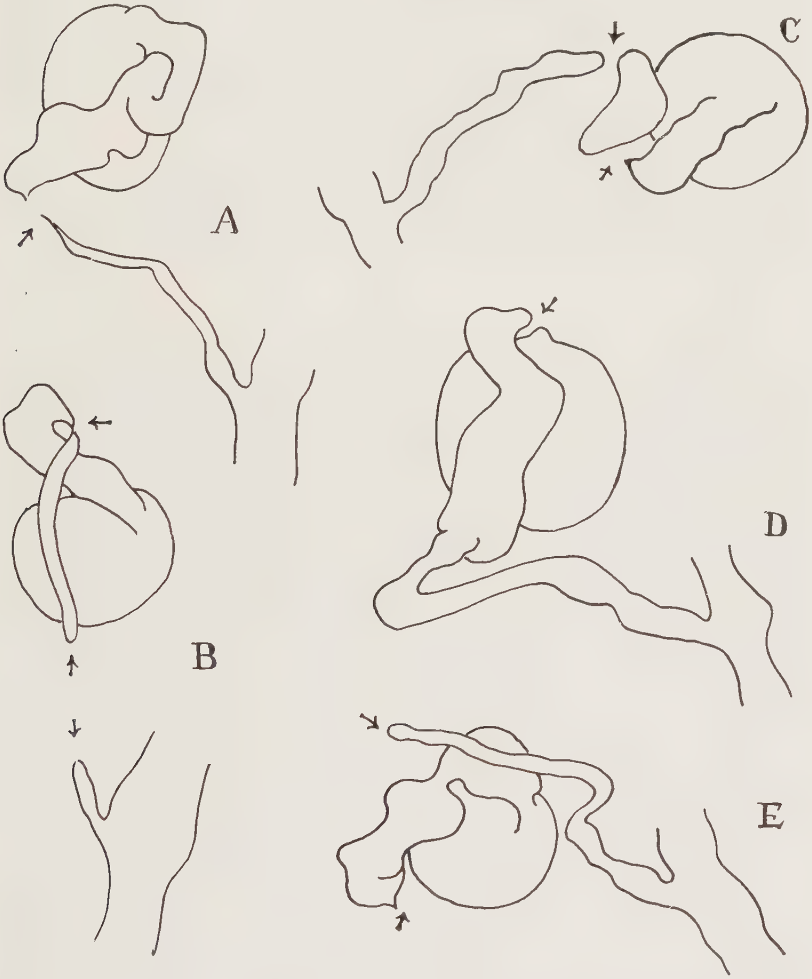

Fig. 3 Reconstructions of broken mesonephric tubules. A and E are broken immediately below the junction of secreting and collecting tubules. B is reduced to a lumenless cord at the junction of the secreting and collecting tubules. In addition, the collecting tubule itself is broken somewhat lower down. C is broken at the junction of the secreting and collecting tubules as well as in the secreting tubule itself. D is broken at the junction of the glomerulus and secreting tubule.

Generally the fragments maintain their relative positions, so that there is no doubt as to which tubule each is derived from, but occasionally the broken portions are widely separated because of either an extensive loss of substance or an actual movement of the parts, probably as a result of pressure exerted by adjacent glomeruli. In such cases it is always possible to determine with certainty the relationship of separated parts by the process of elimination.

There can occasionally be observed a somewhat distended capsule in which the glomerulus is disintegrating in situ, but the last stages of this process are not found in the embryos studied.

DISCUSSION

It has been definitely shown that continuous and apparently healthy wolffian tubules persist up to the 36-mm. stage in man. Numerous observers, including Bremer, Lewis, Shikinami, von Winiwarter, and Wilson, have expressed the opinion, based on histological study, that the human mesonephros is an organ capable of functioning. Felix, on the other hand, believed that it is not, reasoning that since (as he believed) the mesonephric tubules have degenerated completely by the time the 22-mm. stage is reached and the metanephric glomeruli cannot function until about 30 mm., to assume activity on the part of the former would be to assume that the human embryo is left without an excretory organ between the 22- and 30-mm. stages. This line of reasoning is completely invalidated by the evidence presented in this study.

On the other hand, this work does not prove that human mesonephric tubules actually do function as urinary organs. It merely shows that, as far as can be determined morphologically, some of them are capable of doing so. The question is complicated by the fact that there are, as first noted by Bremer, epithelial plates in the chorion of embryos as early as the 29-mm. stage or perhaps even earlier. Bremer has called attention to the fact that these plates and their situation immediately adjacent to capillaries are characteristic of organs in which osmotic interchanges occur. It is therefore quite evident that these structures can perform the function usually ascribed to the renal glomerulus. Indeed, it is fairly certain that they do so, as the exceedingly small size in the human embryo of the reservoir for mesonephric and metanephric urine, the allantois, makes it impossible to believe that the embryonic kidneys actually function.

We thus have a situation in which the mesonephros, capable, as far as can be determined, of secreting urine, apparently remains inactive. The reason for this is not known, but is possibly connected in some way with the level of the blood pressure and the constitution of the blood plasma in the human embryo.

There is some evidence that under suitable circumstances the human mesonephros may actually secrete. Spitzer and Wallin have reported a case of apparent persistence of a pair of actively secreting mesonephroi in a young adult female. Unfortunately, the gross morphological study, which seemed to indicate quite definitely that the bodies in question were mesonephroi, could not be confirmed by histological study, as permission to remove the organs was not obtained. This piece of evidence must therefore also be regarded as inconclusive. However, the sum total of information thus far gathered does seem to favor the view that the human mesonephros actually can function as an excretory organ at one time or another.

The exact nature of this function is unknown. It is probably different from that of the adult amphibian mesonephros, in view of the morphological differences existing in the two forms. Thus the tubule in the human embryo consists of a capsule containing a capillary tuft, a slightly convoluted secretory tubule, and a collecting tubule, while that of the mature amphibian is, according to Huber, divided into capsule, proximal convoluted tubule, straight tubule, distal convoluted tubule, and collecting tubule. The work of White, White and Schmitt, Wearn and Richards, and others has shown that each of these portions has a function of its own.

The human mesonephros, since it differs morphologically from the adult amphibian wolffian body, probably also differs functionally. It is possible that some light can be thrown on the nature of the physiologic activities of the human wolffian body by a careful study of the mesonephroi of such mammals as the opossum, in which the organs persist and function for a considerable period after birth.

SUMMARY AND CONCLUSIONS

1. The mesonephros of the human embryo ten to twelve weeks old and the changes occurring in it are described.
2. It is shown that the mesonephroi of embryos of 31 mm. crown-rump length have fifteen sound tubules, while those of 36 mm. have only ten. No intact tubules were found at 40 or 42 mm. These conclusions are based on evidence derived from reconstructions as well as histological examination of the embryos studied. It is also shown that conclusions as to the persistence of mesonephric tubules which are based merely on the study of isolated histological sections are invalid.
3. The bearing of this evidence on the question as to whether or not the human wolffian body functions is discussed.

This problem was suggested to me by Prof. John Lewis Bremer. I wish to express my gratitude for his aid and encouragement, without which this study could not have been completed.

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THE X ZONE OF THE SUPRARENAL CORTEX IN RELATION TO GONADAL MATURATION IN MONKEYS AND MICE AND TO EPIPHYSEAL UNIONS IN MONKEYS [1]

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The suprarenal cortex of the mouse has been found to contain at certain ages an inner zone with distinctive characteristics. The development and degeneration of this transitory or X zone have been described by the author (Howard-Miller, '27, '29) and by Deanesly ('28). Its development is suppressed by the development of the testis. In the male, if gonadectomized before maturity, or in the normal female, the X zone develops until it constitutes about one-third of the volume of the entire cortex. In the normal female a degeneration is initiated at about the time of the inception of ovulation, and the X zone disappears entirely soon after full growth is attained. The normal disappearance of the X zone in the female is hastened by early pregnancy.

The X zone in the mouse is histologically comparable to a zone in the human suprarenal cortex which develops before birth and degenerates and disappears during the first year of life. These changes were described by Armour and Elliott ('11) and by others.

A comparison of the development and involution of the X zone in man and the mouse suggests the following possibilities: these changes may occur only once in ontogenesis, but at different relative stages of development; or, the

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changes in the mouse may represent specific relations to gonadal activity which may be associated with a reappearance of the X zone at adolescence in man. The second possibility is strengthened by various reports of human suprarenal changes in relation to sex and pregnancy (Aschoff, '24, and Falta, '18), which are not observed, however, in some other species (Kolmer, '12, and Donaldson, '24).

Accordingly, it seemed desirable to test the matter in another primate. Therefore I obtained a series of monkeys to ascertain whether or not the suprarenal reaction could be demonstrated to occur in primates at puberty, in a manner homologous to its occurrence in the mouse, where it occurs for the first time at that period. Since the exact chronological age of the monkeys could not be determined, the sequence of epiphyseal unions has been followed as a criterion of the relative maturity of the animals. This sequence does not necessarily indicate relative age, but in so far as it indicates relative maturity it becomes of greater significance than actual age in a study of development.

In connection with the observations on monkeys, the relations of the gonads to the X zone have been further studied in the mouse.

OBSERVATIONS ON MONKEYS

There was obtained a series of twenty-four monkeys, ranging from approximately quarter-size to full-grown animals. To correlate the condition of the suprarenal with the extent of general development, studies were made of skeletal development and of ovarian and uterine condition. Monkeys were obtained in Venezuela and used in their natural climate, so that possible deleterious effects of a long journey and change of climate were avoided. Twenty-one of the animals were monkeys of uniform general appearance and form, corresponding to Elliot's description of the genus Cebus ('12). Elliot defines several species of Cebus on the basis of the color of fur and the presence of fur crests of various sorts. These monkeys did not appear to belong to a single Cebus group, as they were of various shades of fur, from brown and tan to reddish yellow. None possessed any tufts or crests of fur or any white fur, although, called 'cara blanca' (white face) by the natives. The skin of the face was light-colored, in contrast to the dark-skinned 'cara negra' or Ateleus. Of the remaining three animals, no. 9 differed in having fifteen ribs instead of fourteen, and in being larger than others of its degree of development, but it was otherwise similar to the Cebus group. Nos. 7 and 8 were Ateleus or black-faced spider monkeys.

The degree of skeletal development was studied by means of x-ray plates. The results are given in table 1, together with data on weight, length, sex, and condition of the ovaries. The twenty-four animals are arranged in groups A, B, etc., on the basis of increasing completion of epiphyseal unions. They are numbered serially within the groups. The sequence of union as given in table 1 may be summarized as follows. All epiphyses enumerated in earlier groups are united in later groups.

Group	*Epiphyses united*
A	All of the following epiphyses ununited.
B	Union at the acetabulum, distal centers of humerus, lesser trochanter of femur.
C	Proximal epiphyses of radius and ulna, phalanges of hand, greater trochanter of femur.
D	Phalanges of foot, head of femur.
E	Distal epiphysis of tibia, metatarsals.
F	Metacarpals.
G	Distal epiphysis of femur.
H	Distal epiphysis of ulna.
I	Distal epiphysis of radius, proximal epiphysis of tibia, and head of humerus.

All phalanges of one extremity are grouped together, since no differences were observed among the individual phalanges. Individual exceptions to the order given in this summary are found in nos. 17 and 20 and are detailed in the table.

According to Stevenson ('24), the long bones present a constant sequence of epiphyseal union in man. He includes under this designation the humerus, radius, ulna, femur, tibia, and fibula. He considers the sequence of epiphyseal union

TABLE 1

Epiphyseal unions in monkeys[2]

Development group	A							
Serial no.	1	2	3	4	5	6	7[1]	8[1]
Weight, kg.	0.5	0.53	0.77	0.77	0.85	0.90	3	1.89
Length, cm.	49	44	52	51	52	54	74	62
Corpora lutea	M	M	M	A	M	M	A	A
Skeleton	Prepubertal stage, characterized by a wide separation of the bones at the acetabulum, the presence of all ossification centers studied, and the epiphyses ununited. The ankle was not always observed							

Development group	B						C
Serial no.	9[1]	10	11	12	13	14	15
Weight, kg.	1.12	0.78	0.99	1.43	1.49	0.93	1.63
Length, cm.	56	54	57	64	65	57	65
Corpora lutea	M	A	A	A	A	A	M
Acetabulum	S	S(?)	—	—	—	—	—
Humerus, head	E	E	E	E	E	S	E
Humerus, medial epicondyle	X	E	—	—	—	—	—
Ulna and radius, proximal	E	E	E	E	E	E	—
Phalanges, hand	E	E	E	E	E	E	—
Phalanges, foot	E	E	E	E	E	E	S
Femur, head	E	E	E	E	E	E	E
Greater trochanter	E	E	E	E	E	E	—
Lesser trochanter	E	—	E	E	E	—	—

All specimens listed above showed the following epiphyses ununited: femur, distal; tibia, proximal; tibia, distal; metatarsals; metacarpals; and radius and ulna, distal

All specimens listed below had complete union at the acetabulum, medial epicondyle of humerus, phalanges, and femur, head and greater and lesser trochanters

Development group	D		E	F	G			H	I
Serial no.	16	17	18	19	20	21	22	23	24
Weight, kg.	1.51	1.02	1.76	1.17	2.5	1.86	1.81	1.68	2.0
Length, cm.	63	60	70	68	67	69	70	68	70
Corpora lutea	C	A	C	A	M	M	M	C	C
Humerus, head	E	S	E	E	E	E	E	S	—
Ulna, radius, proximal	—	S	—	—	—	—	—	—	—
Ulna, distal	E	E	E	E	E	E	E	—	—
Radius, distal	E	E	E	E	E	E	E	S	—
Metacarpals	S	S	S	—	—	—	—	—	—
Femur, distal	E	E	S	S	—	—	—	—	—
Tibia, proximal	E	E	E	E	E	S	E	X	—
Tibia, distal	E	E	—	—	—	—	—	X	—
Metatarsals	E	E	—	—	S	—	—	X	—

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[1] Nos. 7 and 8 were Ateleus and larger than Cebus of the same development stage. No. 9 had fifteen ribs.

Nos. 17 and 19 were thin and ill-nourished, and no. 10 had skin lesions. Otherwise the animals were all in good condition. Length was measured from the crown to the tip of the toe in the extended position.

[2] E, epiphysis ununited. S, slight indication of epiphyseal line. —, epiphysis united. C, corpus luteum present. A, corpus luteum absent. M, male. X, not observed.

to be a primitive mammalian character. Dawson ('25) finds certain exceptions to this order of union in the rat. He also finds that the union of the later epiphyses does not occur until old age in the rat. At about three years of age the following epiphyses unite: the olecranon of the ulna, the head, great trochanter, and distal end of the femur, the proximal ends of the tibia and fibula, the distal ends of the radius and ulna, and the head of the humerus. All other epiphyses studied united at or before 158 days of age.

In this group of monkeys the lesser trochanter of the femur unites earlier than the greater trochanter, and the distal epiphysis of the femur unites before the proximal epiphysis of the tibia. These orders are both more in accord with the relations in man as given by Terry ('25) than with those found by Stevenson. On the other hand, the early union of the distal epiphysis of the humerus agrees more nearly with Stevenson's than with Terry's observations. The sequence in these monkeys gives the same order for homologous bones in the knee and elbow. No clear-cut precedence was observed of union at the acetabulum before the distal centers of the humerus or vice versa.

In regard to the rate of epiphyseal union, a rough approximation was all that could be obtained. In some cases the animals had been kept as pets by natives, who gave age estimates possibly accurate to within one or two months. No. 1 was said to be 'three' months old; nos. 5, 8, 13, and 16, 'five to seven months'; nos. 11 and 23, 'six to seven months'; and no. 22, 'eight' months old. The age estimates were from different sources and accord fairly well, within their limits, with the degrees of maturity indicated by the ossification data for Cebus. No. 8 was an Ateleus and probably not comparable with the others in this respect. The Cebus were said to live only about five or six years. Nos. 22 and 23 had nearly complete epiphyseal union, so that there is probably no such delay in epiphyseal union in Cebus as occurs in the rat.

The ovaries of these specimens were of two distinct types. All ovaries which did not contain luteal tissue were small and apparently immature. They contained only small follicles which in most cases were invisible except on section of the gland. Glands of this type were found in all females in development stages A and B.

Ovaries containing corpora lutea were found in females in and beyond stage D except in 17 and 19, which were both underweight compared to others of their degree of skeletal development. Corpora of more than one stage were found in the same gland. No. 23 contained a recently ruptured follicle half filled with a blood clot, a large corpus luteum filled with closely packed cells, and a smaller corpus luteum in which the cells were widely separated by invading ovarian stroma, so that in this case three separate ovulations are probably represented. Since the corpora lutea last from one ovulation to the next, after ovulation has been initiated one would expect to find corpora lutea of some stage always present, provided that ovulation continues at the rate indicated in this set of ovaries.

Sexual maturity as indicated by the first ovulation may therefore be said to occur at least as early as stage D, the beginning of the union of the epiphyses of the femur and after fusion at the acetabulum, probably at an age of six to eight months. This degree of skeletal development corresponds to seventeen to eighteen years in man, according to Stevenson.

The uterus of no. 23, which had recently ovulated, had a well-developed endometrium with fairly high epithelium, but with no trace of vascular congestion. Nos. 16 and 18 had blood macroscopically evident in the lumen of the uterus, but no trace of blood externally or in the vagina. Microscopically, the endometrium of nos. 16 and 18 contained a large amount of extravasated blood, and epithelial desquamation was evident, especially in no. 16. Cebus therefore undergoes uterine menstrual changes, although external bleeding was not observed in these animals.

If the semi-annual oestrous is more primitive than the polyoestrous condition, one might expect to find, in the lower primates such as Cebus, either a semi-annual ovulation or some form of intermediate condition between it and the more closely recurring ovarian and uterine changes of the higher primates. In Venezuela it is the popular belief that the birth of monkeys is related to the rainy seasons, which occur semi-annually in May and June and in November and December in the region where these animals were obtained. This suggests the possibility of semi-annual breeding seasons. The females of this series were obtained during the latter part of April and the first week in May, 1928. If there is seasonal ovulation, for which I have no evidence, these ovaries suggest that it is characterized by a series of ovulations rather than by the maturation of a single follicle.

The early completion of epiphyseal union in Cebus indicates a much closer general correspondence between the duration of skeletal development and the period of gonadal function in Cebus and man than between primates and rats: that is, there is no delay in epiphyseal union relative to the period of gonadal function in primates, as there is in the rat. However, Long and Evans ('22) find that in the rat the first ovulation occurs at an average of seventy-seven days (range, 45 to 147). Dawson finds that in the rat the union of the epiphyses of the head of the radius and of the distal tibia and fibula occurs between 83 and 130 days of age, so that the inception of ovulation and the beginning of epiphyseal union are approximately simultaneous in rats as in primates, even though the completion of union is delayed until relative old age in the rat.

On the other hand, an example of a developmental process which is not synchronized with skeletal development in primates is found in the eruption of teeth. In many of the younger monkeys unerupted canines and incisors were present. In all of these animals skeletal development had proceeded to a stage corresponding to that of a human of thirteen to fifteen years, so that the relative timing of these two processes is not the same in the two primates.

The form of the suprarenal gland is not the same in Cebus and Ateleus. In Cebus it is composed of triangular flattened lobes and resembles the human suprarenal in form. In Ateleus it is nearly spherical without lobes, approaching the mouse gland in type, and the medulla is much larger, in proportion to the cortex, than in Cebus. In both cases the width of the cortex from the external edges of the zona glomerulosa to the zona reticularis (medullary border) is not much greater than the width of the cortex in the mouse. This suggests that there is a tendency to maintain a certain ratio between the surface of the cortex and its width. If so, the differences in the relative amount of medulla would account for the differences in shape in Cebus and Ateleus, for with the large amount of medulla the ratio between surface and width can be maintained without the production of flattened lobes.

Histologically, the suprarenals of the monkey presented no variations analogous to that described for the mouse during the same stages of gonadal development. At all stages the cortex of the monkey was comparable in general structure and staining reaction to that of the adult mouse after the transitory zone has disappeared. Therefore, if a homologous reaction occurs in the monkey, it must be completed at an earlier stage than that at which it starts in the mouse, considered in relation to gonadal development.

These monkey suprarenals contained no clear-cut differentiation into reticular and fascicular zones. A uniform type of cells extends from the glomerular zone to the medulla. In frozen sections stained in sudan III after formalin fixation, this area stains a uniform reddish-orange color.

Nos. 10 and 14 differed from the rest in possessing scattered vacuoles the size of a single cell. This vacuolization extended irregularly throughout the cortex, and was not similar in character to the vacuolization of the transitory zone in the mouse. The two animals showing this were thin and ill-nourished; the fur of no. 10 was in poor condition and skin lesions were present. The animals had both been kept in captivity. The possibility exists that the suprarenal condition was a response to infection or an evidence of malnutrition.

THE EFFECT OF GONADECTOMY ON THE SUPRARENAL CORTEX OF THE MOUSE

The effect of gonadectomy on the visible structure of the suprarenal cortex of the mouse varies according to the age at which the gonads are removed. The effects in the two sexes are different and will be considered separately.

In the case of the male, as I have previously ('27) reported, there occurs a marked hypertrophy of the X zone if the gonads are removed before puberty. The present paper includes results of a study of the fate of such an X zone in the male. Nine males were castrated at ages between twelve and twenty-eight days. They were killed at ages of 231 to 266 days, and the rudimentary condition of the seminal vesicles verified. The suprarenals in all cases contained remnants of X-zone tissue, either empty reticular tissue in the inner part of the cortex or a narrow compact band of small flattened cells. In two mice castrated at twenty-four days, the left suprarenal was obtained at 160 days and the right gland at 199 days. In both cases the younger gland contained a large X zone which had not started to degenerate, while the older gland showed a smaller zone of small compact cells and some empty reticular tissue. It is concluded that castration does not prevent the ultimate degeneration of the X zone, although it results in an initial increased growth of the zone. The X zone of the male castrated before puberty thus develops and subsequently degenerates, as does the zone in the normal female.

Sixteen males castrated after puberty were not observed to show a return of the highly developed reticular zone which is designated as the X zone; but, after intervals of fourteen to 175 days, the inner part of the cortex shows irregular changes which tend to make the characteristics of the typical zona reticularis more prominent. The differentiation is too indefinite to be of value at present, but is not comparable to the X zone which results from castration before puberty.

In the female I have failed to observe any effect of the removal of the ovaries upon the structure of the suprarenal cortex. The changes which normally progress in the cortex are unaffected by the absence of the ovaries. In addition to the experiments reported in 1927, I have studied a group of eleven females, litter mates of the group of nine males discussed above, that were similarly gonadectomized between the ages of twelve and twenty-eight days. At the ages of 231 to 266 days, complete disappearance of the X zone was observed in six cases, while the others showed small remnants of degenerating X zone. The females when killed were mostly older than the males, so that the complete disappearance of the X zone in the females and not in the males may not be indicative of a slower degeneration in the males.

DISCUSSION

It has been found that if mice of both sexes are gonadectomized before puberty, the X zone ultimately degenerates, as it does in unoperated mice, showing that this reaction of the suprarenal may take place without any stimulus received from the mature gonads. The degeneration starts in the female mouse at the time of gonadal maturation, but not because of it. The mouse, in comparison to man, shows a retardation of the suprarenal reaction in relation to gonadal maturation. The temporary sex differences which the suprarenal cortex shows in normal mice are apparently secondary interference effects resulting from a telescoping together of various phases of ontogeny in the rapidly developing mouse, rather than being essentially related events.

It was found that monkeys failed to show such suprarenal reactions during puberty. This is regarded as entirely in accordance with the experimental results in the mouse which indicate that the association of the suprarenal reaction with puberty in that species is a result of factors other than specific interendocrine reactions. It is unfortunate that I have been unable to secure newborn monkeys, as it would be desirable to determine whether the X zone develops and degenerates at birth as it does in man. However, the facts that the X zone is not related to birth in the mouse, and is not related to puberty in the monkey, show that it is not necessarily associated with either of these physiological events. The evidence suggests that the development and degeneration of the X zone resembles gonadal maturation in that it appears to be an endocrine reaction which occurs once in the lifetime of an individual and as a result of inherent developmental forces of the organism. While I have no explanation for the relative age differences in the suprarenal reaction in man and the mouse, I feel that this asynchrony is probably not essentially different from other instances of asynchrony in the development of mammalian organ systems, a few examples of which which have been mentioned above. The histological sex differences in mice, while secondary to the development of the gland, may be, however, an expression of fundamental suprarenal cortex-gonad relationships which do not obtain similar manifestations in all species.

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CONCLUSIONS

The X zone of the suprarenal cortex undergoes degeneration in normal mice and in those of both sexes which have been gonadectomized before puberty, indicating that this reaction of the cortex is essentially independent of gonadal maturation.

The suprarenal cortex in monkeys shows no changes in relation to puberty analogous to those occurring in the normal mouse. This is offered as further evidence that the relation observed in the mouse is not an essential one.

The inception of ovulation in monkeys coincides approximately with the beginning of the process of epiphyseal union. The sequence of epiphyseal union in Cebus monkeys has been found to coincide, with minor differences, to that in man. No structure resembling the X zone was observed in the adrenal during the progress of epiphyseal unions.

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UTERUS AND FETAL MEMBRANES OF THE INDIAN ANTELOPE (ANTILOPE CERVICAPRA)

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THREE TEXT FIGURES AND THREE PLATES (TWELVE FIGURES)

Detailed studies on the placentation of ruminants have been in most instances limited to those forms readily procurable from the slaughter houses, namely, the cow and the sheep. The placentation of the sheep has been the subject of extensive investigations and with these investigations one associates the names of Bonnet, Kolster, Turner, and Assheton. The true ruminants, or Pecora, possess a type of placentation that is not found in any other group of mammals. It is characterized by aggregations of villi on the fetal placenta with corresponding aggregations of crypts on the uterine wall for the reception of the fetal villi. This combined structure, made up of maternal and fetal elements, has been called a 'cotyledon' or 'miniature placenta.' The number, arrangement, and structure of the cotyledons constitute a basis of classification of ruminants. We may find in the works on the classification of mammals statements made that such and such a number of cotyledons is characteristic of a group of ruminants, even though some of the members of the group may not have been studied at all. However, it so happens that the assumptions made by the taxonomist on the basis of several characters of a few members of a group are true in many cases for the remaining members of the group.

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[1] The author takes great pleasure to thank Dr. R. P. Cowles for his kindness in offering many helpful suggestions in the preparation of this paper.

Specimens obtained from museums, from zoölogical gardens, or from expeditions have been the main source of material concerning the placentation in forms other than the cow and the sheep. Study of these more or less rare specimens has been in general carried on from a taxonomic point of view only, probably because the material was inadequately preserved for the investigation of histological details.

Of the twelve sections comprising the family Bovidae, the placental characters of members of five sections have as yet not been described, i.e., Cephalophine, Neotragine, Oreotragine, Antilopine, and Ovibovine. Through the courtesy of Dr. H. Fox, of the Zoölogical Society of Philadelphia, opportunity has been afforded for the study of the fetal membranes and uterus of the Indian antelope (Antilope cervicapra) and the results of a study of these are embodied in this paper. The Indian antelope belongs to the antilopine section (Flower and Lydekker, '91) and there is only one existing species of the genus Antilope. The placental characters have heretofore been undescribed. Judging from its taxonomic position, certain placental characters might be predicted, namely, a large number of cotyledons and in general a type of placentation similar to that found in the sheep.

Before describing the uterus and fetal membranes of the Indian antelope, let us consider briefly placental classification.

Turner ('75) used the distribution and form of the villi on the fetal placenta as the basis for his classification. On the chorion of one of the primitive ungulates, i.e., the pig, the villi are uniformly distributed over the entire surface, with the exception of a small area at the ends or poles. The villi are short and simple and fit into shallow crypts. This type of placentation was designated as 'diffused.' The villi on the chorion of the ruminants are aggregated in groups or tufts with intervening bare spaces. They are arborescent and much longer than the villi on the 'diffused' type of placenta. In addition, the tufts of villi fit into a corresponding group of tortuous crypts on the uterine wall. Turner termed this type of placentation 'cotyledonary.'

Grosser ('09) classified placentae according to physiological types. Each type has a different degree of separation of the maternal blood stream from the fetal blood stream. The 'diffused placenta' of Turner is the 'epithelio-chorialis' type of Grosser, since the maternal tissue is composed of epithelium, connective tissue, and capillary endothelium. The uterine epithelium is contiguous with the chorionic epithelium, hence 'epithelio-chorialis.' Separation of the fetal membrane from the uterine wall occurs readily. The 'cotyledonary placenta' is the 'syndesmo-chorialis' type. During gestation the uterine epithelium degenerates (with the exception of the epithelium of the uterine glands) and a very highly vascular modified subepithelial connective tissue is in apposition with the chorionic epithelium. Back of the chorionic epithelium is the endothelium of the fetal capillaries. The fetal portion of the placenta separates with difficulty from the maternal portion, the villi being tightly locked into the maternal crypts.

Briefly, the fundamental differences between the 'epithelio-chorialis' or 'diffused' and the 'syndesmo-chorialis' or 'cotyledonary' types are the following: In the first type the villi are rudimentary and distributed over the entire surface of the chorion, whereas in the other type the villi are aggregated into tufts and are far more complex. Separation of the maternal and fetal elements of the placenta occurs readily in the 'diffused' placenta and with difficulty in the 'cotyledonary' placenta. The placenta of the Indian antelope which will be described belongs to the 'syndesmo-chorialis' or 'cotyledonary' type.

UTERUS AND FETAL MEMBRANES OF INDIAN ANTELOPE

The specimen obtained was fixed and preserved in 5 per cent formalin and contained complete internal female genitalia (fig. 4). The relationship of the fetal membranes to the uterine wall was well preserved and at the time of fixation the right horn of the uterus, which contained the embryo, was slit open. The embryo (124 mm. crown-rump measurement) lay with its back opposite the antimesometral border of the uterus, facing the fundus. Gross antelope characteristics could be recognized in miniature in the embryo: slender, delicate legs; fore legs slightly shorter than the hind legs, and long neck with typical antelope head (fig. 7). In the region of the insertion of the umbilical cord the surface of the amniotic membrane was studded with numerous papilliform cysts of various sizes. A typical allantochorion characteristic of the cotyledonary (syndesmo-chorialis) placenta was found (fig. 2).

Uterus

The uterus was of the bicornuate type (fig. 1). From external examination it seemed that only one horn was gravid, since one was very much dilated and the other had the appearance of a normal non-gravid horn. The ovaries were two well-defined, almost spherical organs, of about equal size, with a visible corpus luteum in the left ovary, and the horns were separated by an incomplete septum situated in the corpus uteri. The allantochorion extended through both horns, while the amnion was limited to the gravid horn. The fallopian tubes were short and very convoluted, terminating in a relatively large fimbriated funnel.

As is typical of the ruminants, the uterine surface of both horns of the Indian antelope was interrupted by spongy prominences, the maternal cotyledons (fig. 9). Von Baer (1828) called these spongy uterine prominences 'Mutter-Kuchen,' while the tufts of villi on the chorion were called 'Frucht-Kuchen.' A total of ninety-six spongy prominences or maternal cotyledons were found in the two horns. The left horn contained forty-two and the right horn, fifty-four. Two structural types of maternal cotyledons were found (fig. 3): those that were mere prominences on the uterine wall and those that were attached to the uterine wall by peduncles or stalks. These two types have been designated as 'non-pedunculated' and 'pedunculated' maternal cotyledons. Kolster ('08) described and figured two types of maternal cotyledons for the reindeer (Rangifer tarandus), i.e., those that were pedunculated and those that were non-pedunculated. Those with the smallest diameters were non-pedunculated and may be considered as the so-called 'accessory cotyledons' of Turner ('78). The significance of the term 'accessory' as applied to cotyledons is not clear; the term was used perhaps to indicate that these cotyledons were not as important functionally by virtue of their size. The non-pedunculated cotyledons varied from 1 to 16 mm. in diameter. Most of the smallest cotyledons were convex, but some have been found that were somewhat concave. The pedunculated cotyledons varied from 5 to 12 mm. in diameter; the majority of them were 8 mm. in diameter. No cotyledons were found in the extreme tips of the horns (about 2 cm. from the end), and none were found on the wall of the body of the uterus.

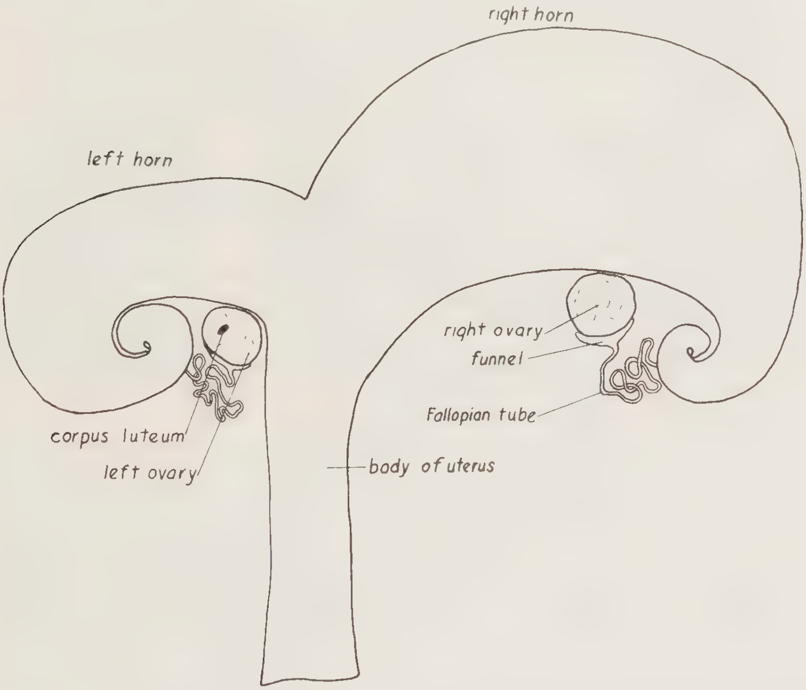

Fig. 1 Outline drawing of uterus of Indian antelope (from dorsal aspect).

The maternal cotyledons in the right or gravid horn were distributed irregularly in rows more or less parallel to the long axis of the horn. Beauregard and Boulart ('85) (Oryx algazelle) have reported that the maternal cotyledons were arranged in rows parallel to the mesometral border. This arrangement obtains in general in the Bovidae (Andresen, '27). The cotyledons present on the uterine wall dorsal to the embryo were in general larger than those present on the uterine wall ventral to the embryo. In this horn there were twenty pedunculated (varying from 5 to 15 mm. in diameter) and thirty-four non-pedunculated cotyledons (varying from 1 to 16 mm. in diameter). Of the nine cotyledons under 4 mm. present in the gravid horn, seven were found in close proximity to, and on, the incomplete intercornual septum. A non-pedunculated cotyledon was present on the free edge of the septum facing the cavity of the body of the uterus.

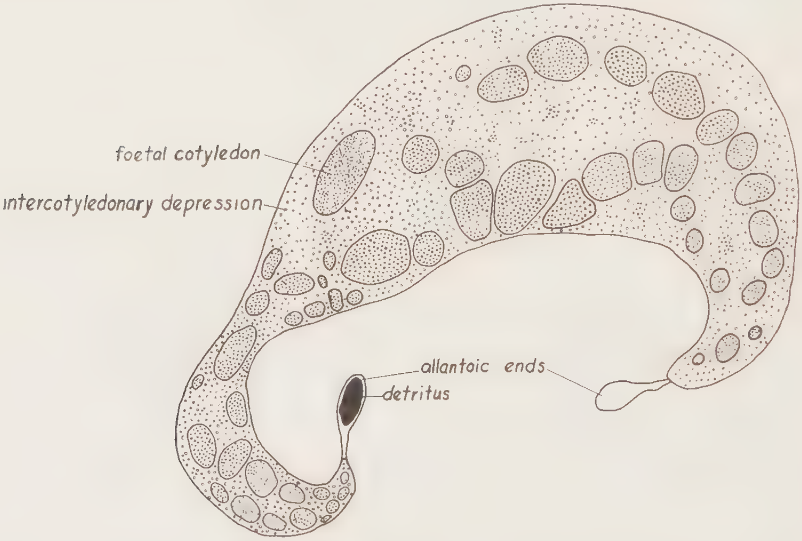

Fig. 2 Outline drawing of the outer surface of the allantochorion spread out, showing the arrangement and shape of the fetal cotyledons, intercotyledonary depressions, and the allantoic ends (diverticulae allantoides).

The cotyledons in the left horn were arranged in four regular rows parallel to the mesometral border. The mesometral border divided the four rows into two groups of two rows: two rows of cotyledons on the dorsal wall and two rows of cotyledons on the ventral wall of the horn of the uterus. Only six cotyledons in the left horn were non-pedunculated and these were all convex. The cotyledons in the rows closest to the mesometral border were mostly pedunculated cotyledons (twenty pedunculated and two non-pedunculated). In the two rows distal to the mesometral border there were sixteen pedunculated cotyledons and four non-pedunculated cotyledons. Cotyledons closest to the mesometral border were in general smaller than the more distal ones. The non-pedunculated cotyledons in this horn varied from 5 to 9 mm. in diameter, while the pedunculated cotyledons varied from 3 to 7 mm. in diameter. The convexity of the cotyledons was so marked that they presented the appearance of domes or spheres suspended from the uterine wall by stalks or peduncles. Due to this marked convexity, the cotyledons almost obliterated the cavity of the horn. They differed from the cotyledons in the gravid horn in the degree of convexity

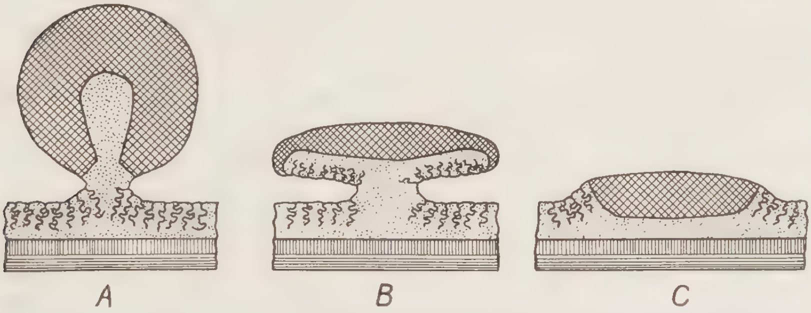

Fig. 3 Diagrams of sections of types of maternal cotyledons. Cross-hatched areas indicate extent of cotyledonary labyrinth, dark serpentine lines indicate distribution of uterine glands. A is a spherical pedunculated cotyledon found in the left horn only (non-gravid). B is a pedunculated cotyledon found in the right horn only (gravid). (Note distribution of uterine glands.) C is a typical non-pedunculated cotyledon, occurring in both horns.

(those in the gravid horn had a much decreased convexity and in some instances a slight concavity).

Whether or not the presence of the embryo in this specimen in the right horn and the structural differences of the cotyledons as compared to those in the left horn were a coincidence or the rule cannot be settled on the basis of a single specimen. This difference in types of cotyledons provokes an interesting question in development: Were the types of cotyledons discussed fixed structures and characteristic of the organ or effects of conditions (increase in, *a*) the fluid content of the allantochorion, *b*) the content of the amniotic sac (embryo and amniotic fluid)) arising during development of the embryo?

The portion of the uterine surface between the cotyledons or 'intercotyledonary area' (so-called by Assheton, '06) was covered with numerous small, almost circular depressions, the dilated mouths of the uterine glands (fig. 9). These depressions, by virtue of their position, have been called, for convenience, 'maternal intercotyledonary depressions.' They were distributed irregularly, averaging 375 per square centimeter (ten counts were made at random through both horns) and varied from 110 μ to 380 μ in diameter (fifty measurements at random through both horns). They extended to the extreme ends of both horns. This pitted surface was thrown into small irregular folds.

All types of maternal cotyledons were made up of a network of thin, highly vascular lamellae giving rise to a labyrinth of tortuous crypts (fig. 9). Superficially, the lamellar network presented a spongy consistency and a honeycomb appearance. The cypts in the cotyledons of smaller diameter (1 to 3 mm.), however, were shallow and non-tortuous. Intercotyledonary depressions were found at the periphery of the spongy region of the non-pedunculated cotyledons and on the under surface and stalk of the pedunculated cotyledons. Large branches of the main uterine artery and vein were present at the base of each cotyledon. These immediately subdivided into smaller vessels and capillaries, forming a vascular tree. No decided superficial pigmentation of the maternal cotyledons was observed. Bonnet ('80) reported that the maternal cotyledons as well as the intercotyledonary area in the sheep may under some conditions become heavily pigmented, but that excessive pigmentation did not occur generally, since from 200 specimens of pregnant uteri only fourteen showed widespread pigmentation.

No trace of uterine epithelium was found on the cotyledonary lamellae. The epithelium may possibly have been stripped off when the villi were withdrawn from their corresponding crypts, but more probably at this stage of placental development it had degenerated. The cytoplasm as well as the nuclei of the connective tissue making up the vascular lamellae stained heavily (either with Mallory's connective-tissue stain or Delafield's haematoxylin). As already mentioned, the lamellae were rich in capillaries of the uterine vessels, and in many places the capillary endothelium was bare and ruptures were common. Extravasated blood was present in the crypts, suggesting a lacunar circulation (this condition has been observed by Assheton, '06, for the sheep). Groups of dark brown spherules were found irregularly dispersed through the extravasated blood. These spherules were taken to be pigment granules resulting from the degeneration of extravasated maternal red blood corpuscles. They were not affected by stains. The connective tissue immediately back of the cotyledonary labyrinth was compact and densely nucleated and the connective-tissue fibers were thin and very little ground-substance was present. The nuclei were elliptical and stained lightly. Back of this compact layer came a layer of loose connective tissue, the fibers of which were thick and widely separated by ground-substance. The upper portions of the tubular uterine glands were embedded in this loose connective tissue. No glands, however, opened into the cotyledonary labyrinth.

The epithelium was absent from the intercotyledonary area (fig. 10). It is of interest to note that during early stages of gestation in Rangifer tarandus, the uterine lumen is lined with a layer of cylindrical epithelium, but that in the later stages it degenerates and the compact layer of the submucosa comes in contact with the chorionic epithelium (Kolster, '08). The connective tissue of the submucosa was stratified into two layers: a compact, heavily staining layer and a loose, lightly staining layer. In the darkly staining layer the nuclei were elliptical, heavily stained, closely packed, and definitely oriented with respect to the axis of the horn. The connective-tissue fibers were coarse, heavily stained, and closely matted together, with very little intervening ground-substance. Rich capillary vascularization was present in the compact layer and frequently the capillary walls were ruptured. In the lightly staining underlying layer, the cells were considerably scattered and stained less heavily. The connective-tissue fibers were finer and lightly stained. The intercellular ground-substance was abundant. No pigmentation was observed either in the compact or loose submucosal tissue as described and figured by Kazzander ('90) in the sheep. Outside of these two differentiated connective-tissue layers were the two rather thick muscle layers.

The uterine glands penetrated deeply into the submucosal tissue. They were convoluted, branched tubular glands, and were enmeshed in a compact capillary network. The lumen of each gland was lined with a cuboidal epithelium which terminated at the mouth of the gland (fig. 11). This epithelium possessed elliptical, heavily stained nuclei and granular cytoplasm. The cells at the mouth of the gland stained heavily and had the appearance of being degenerate. In many places the glandular epithelium together with endothelium of the uterine capillaries was ruptured and extravasated blood was found in the lumen. The lumen of the gland near the mouth was filled with a coagulum and cell débris, and was considered by earlier investigators as a pabulum or food for the embryo (Assheton, '06, and Bonnet, '82). In this pabulum were identified maternal red blood cells, leucocytes, degenerating epithelial cells, brown pigment granules, and a mucus-like ground-substance. These are constituents typical of uterine milk described by Bonnet ('82), Assheton ('06), and others. It is commonly supposed that the pigment granules in the uterine milk have their origin from the haemoglobin of degenerating maternal red blood corpuscles. This pigment is undoubtedly absorbed by the chorionic epithelium and was considered by earlier investigators as a source of iron for the developing embryo. In the specimen of the Indian antelope, however, not all pigment was associated with uterine milk, since small masses were found in the maternal cotyledons.

Fetal membranes

The allantochorion of the Indian antelope was found to be an irregularly crescentic sac that was very much dilated in the right horn of the uterus and quite small in the left horn (fig. 2). In those ruminants in which each uterine horn contains an allantochorion, the embryo is centrally located (or nearly so) in the allantoic sac (sheep, Assheton, '06). In some instances the embryo is decidedly eccentrically situated, as is the case in the Indian antelope (Kolster, '08, and Cervicapra bohor, Sedlaczek, '12). When this condition obtains, the portion of the allantoic sac that contains the embryo and its amnion is found in the enlarged horn of the uterus (usually the right horn), and this condition was found in the Indian antelope. Owing to the fact that the maternal cotyledons were spherical and almost obliterated the cornual lumen of the left horn, the allantoic lumen was encroached upon and only an irregular slit remained. The amniotic sac was contained in the right horn and almost completely filled it. The ends of the allantochorion were pushed out into thin-walled diverticula. These were filled with fluid and a brownish detritus. Differences have been found to exist between groups of ruminants with regard to the presence or absence of diverticula at the ends of the allantochorion. In the Cervidae these structures are lacking, while in the Bovidae they are present. Turner ('75) described these structures for the pig and sheep and called them 'diverticulae allantoides.' He considered them as protrusions of the allantoic sac beyond the chorion. Jenkinson ('06), however, studying the allantochorion of the sheep, concluded that the so-called 'diverticulae allantoides' were not protrusions of the allantoic sac beyond the chorion, but that they were the degenerate ends of the chorion. No light can be thrown on this disputed point from a single specimen of the Indian antelope.

Scattered over the surface of the chorion, patches of villi, the fetal cotyledons, were found. These were of varying diameters and patterns corresponding to the size and form of the maternal cotyledons. The fetal intercotyledonary area was free from villi and thrown into small irregular folds fitting into the folds on the maternal intercotyledonary area. In addition, small brownish circular patches were scattered over this surface (fig. 6), except in the region of the diverticulae allantoides. These brownish patches were designated as the fetal 'intercotyledonary depressions.' The inner surface of the amniotic membrane around the umbilical cord was studded with whitish cysts of various forms and sizes.

There were two types of fetal cotyledons: concave and flat (fig. 6). The concave ones were the fetal counterparts of the pedunculated maternal cotyledons, and could not be separated from them without seriously tearing the fetal cotyledons. It may possibly be that the structure formed by the concave fetal and pedunculated maternal cotyledons serves secondarily to hold the fetal membranes more rigidly in position. The flat fetal cotyledons could be separated more readily than the concave ones. Preponderance of concave fetal cotyledons is a placental characteristic of the family Bovidae (Andresen, '27).

Whether the fetal cotyledons were concave or flat, difficulty was experienced in separating them from the maternal cotyledons and any attempt to pull the villi from the maternal crypts resulted either in tearing the cotyledonary lamellae or the pulling off of a villus. The villus was made up of a tuft of elongated cylindrical processes (like a miniature paint brush). There was no lateral branching at the ends of the processes, all branching took origin from the main stem near the base. The middle portion of one of these cylindrical processes was thicker than at the free end or at the origin. Thus the villus process was virtually locked into its corresponding maternal crypt. The more centrally located villi were the longest and the peripheral ones the shortest. The mesenchymatous tissue beneath the fetal cotyledons was considerably thickened and microscopic examination showed that there was a large number of nuclei present in this region. Mesenchymatous tissue ramified by fetal capillaries made up the core of the villi. The capillaries were closely applied to the bases of the epithelial cells covering the villi, but there was no invasion of epithelium by capillaries, as has been reported for the chorionic epithelium of the pig (Tafani, '87; Goldstein, '26; and Heuser, '27).

The villi were covered with two types of epithelium: squamous and columnar. At the tips of the longer villi, the epithelium was decidedly flattened and almost syncytial, the nuclei darkly stained, and the cytoplasm was very granular. At the base of the villi the epithelium was columnar (five or six times as tall as the epithelium on the tip). These are the so-called 'arcades' (sheep, Assheton, '06). Between the tip and the base of a villus there was a gradual transition from squamous to columnar. The epithelium on the tips of the centrally located villi was essentially squamous, while on the tips of the peripheral ones it was cuboidal. No inclusions such as crystals or pigment granules were found in any of these cells.

The fetal intercotyledonary area was covered with small depressions, the fetal counterparts of the depressions on the uterine wall. Von Baer (1828) and Turner ('76) reported small polygonal areas on the chorion of the cow that were opposite the openings of the uterine glands. Assheton ('06) observed 'little rings of raised tissue' on the fetal intercotyledonary surface of the sheep. Thus there is present on the chorion of some ruminants a gross morphological differentiation that is probably analogous to the more complex structure ('rosette,' 'areola') found on the chorion of the pig.

The chorion was covered with a layer of columnar epithelium, and the cells in the intercotyledonary depressions (fig. 12) were taller than the surrounding ones (about twice as tall). A coagulum was found in the depressions in which there were maternal leucocytes, degenerating red blood corpuscles, pigment spherules, and a mucus-like ground-substance. Pigment spherules and rod-like crystals were found in the cells, undoubtedly indicating that these had been absorbed from the pabulum. The cells surrounding the intercotyledonary depression contained fewer pigment spherules and crystals. The ends of the cells were frayed, the cytoplasm granular, and the nuclei poorly differentiated. Bonnet ('82), Assheton ('06), and Jenkinson ('06) have reported the presence of rod-like crystals and fat globules in the chorionic epithelium.

Bernard ('59) described whitish bodies on the amnion of the sheep and found that during gestation these bodies develop and that at birth they atrophy. The name 'placques hepatiques' was given to them because they were supposed to carry on functions similar to the liver. Bernard found glycogen only in the amniotic cysts. Turner ('79) described similar bodies projecting into the amniotic sac of the hog deer (Cervus porcinus). The amniotic cysts in the Indian antelope were of two general shapes: fungiform and filiform (fig. 8). They were in no way connected with the fetal capillary system, their free ends being bathed by the amniotic fluid. Examined under low magnification, an amniotic cyst appeared to be a solid opaque shell surrounding a less opaque core. Microscopically, it was found to be made up of stratified epithelium. Red-staining inclusions were found in the peripheral cells near the tip (using Mallory's connective-tissue stain). The nuclei were poorly differentiated (figs. 14 and 15).

SUMMARY

From the foregoing description it is seen that the Indian antelope (Antilope cervicapra) belongs to the family Bovidae in having the following placental characteristics:

1. A large number of cotyledons (ninety-six).
2. Two types of fetal cotyledons (flat and concave).
3. Presence of diverticulae or allantoides.
4. Presence of cysts on amnion and umbilical cord.
5. Fetal intercotyledonary area differentiated into depressions.
6. Uterine glands well developed.

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PLATE 1

EXPLANATION OF FIGURES

4 General view of specimen as obtained. The gravid horn is slit open, exposing the embryo. × 0.6. *al.ch.*, allantochorion; *u.c.*, umbilical cord; *gr.hn.*, gravid horn; *n.gr.hn.*, non-gravid horn; *corp.lut.*, corpus luteum; *ov.*, ovary; *ur.bld.*, urinary bladder.

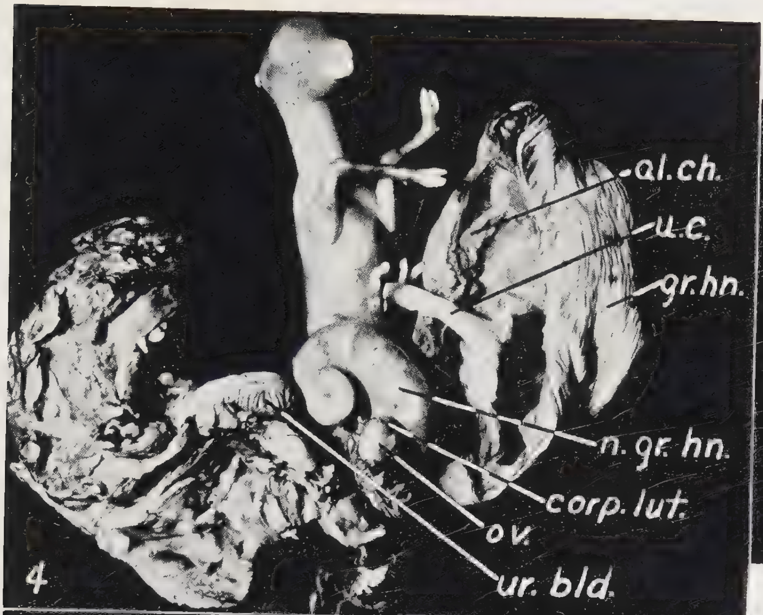
5 Two uterine horns with embryo removed, showing gravid horn slit open, looking into the amniotic cavity with the allantochorion in position. × 0.8. *al.ch.*, allantochorion; *ov.*, ovary; *mat.cot.*, maternal cotyledon; *n.gr.hn.*, non-gravid horn; *u.c.*, umbilical cord; *fet.cot.*, swollen connective tissue back of fetal cotyledon.

6 Surface view of flat fetal cotyledon. × 5. *fet.cot.*, fetal cotyledon showing villi; *fet.int.dep.*, fetal intercotyledonary depression.

7 Surface view of right side of embryo. × 0.4.

8 Surface view of portion of amnion showing two types of cysts: fungiform and filiform. × 50. *am.cy.*, amniotic cyst.

9 Surface view of non-pedunculated maternal cotyledon. × 4. *mat.int.dep.*, maternal intercotyledonary depression (note large numbers of maternal intercotyledonary depressions); *cot.lab.*, cotyledonary labyrinth.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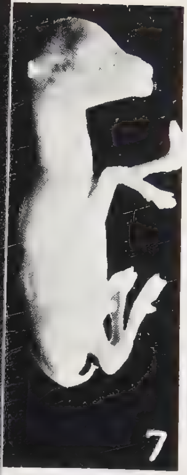
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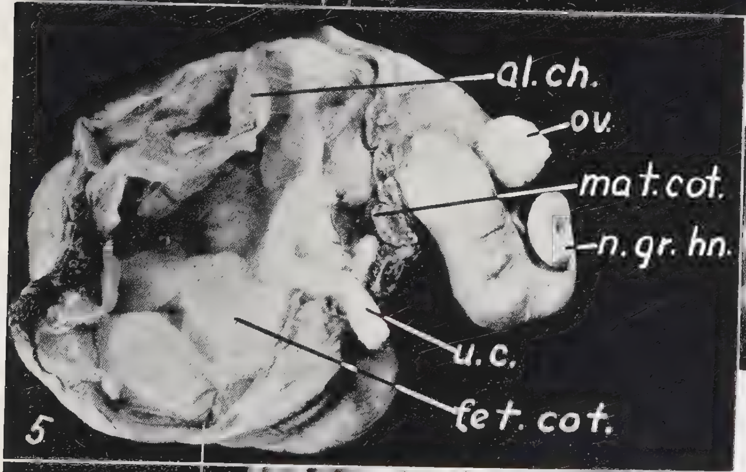
al.ch.
ov.
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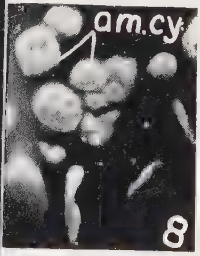
u.c.
fet.cot.
5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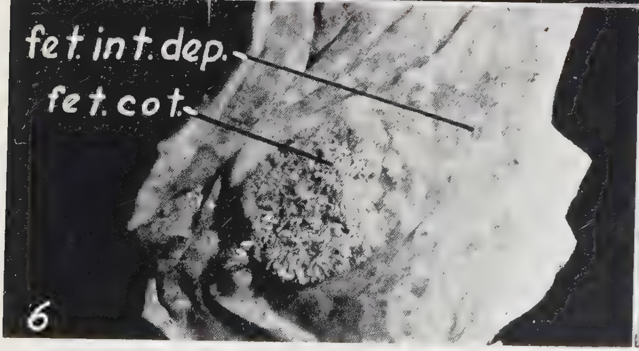
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fet.cot.
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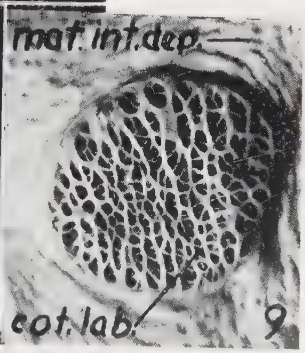

mat.int.dep.
cot.lab.
9

PLATE 2

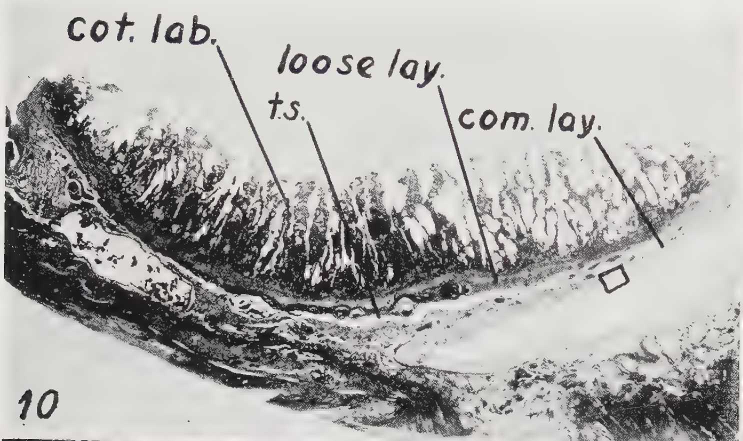
EXPLANATION OF FIGURES

10 Transverse section through a partly pedunculated maternal cotyledon. × 15. *cot.lab.*, cotyledonary labyrinth; *loose lay.*, loose connective-tissue layer; *com.lay.*, compact connective-tissue layer; *t.s.*, tear in section.

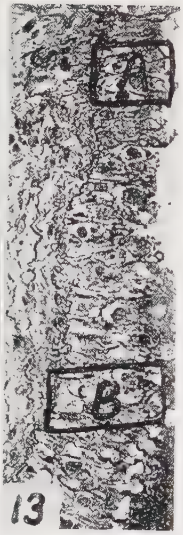
11 Section through maternal intercotyledonary depression, showing uterine-gland opening. × 300. Figure 11 is a higher magnification of the square indicated on figure 10. Note absence of epithelium on uterine surface. The dark spots are maternal erythrocytes. *ut.gl.*, uterine gland; *pab.*, pabulum; *gl.mo.*, mouth of uterine gland.

12 Section through fetal intercotyledonary depression. × 300. *pab.*, pabulum; *fet.ep.*, fetal epithelium; *con.tis.*, connective tissue.

13 Section through lip of fetal intercotyledonary depression, showing difference in height of epithelium. × 300. Compare areas A and B.


cot. lab.
loose lay.
t.s.
com. lay.

10



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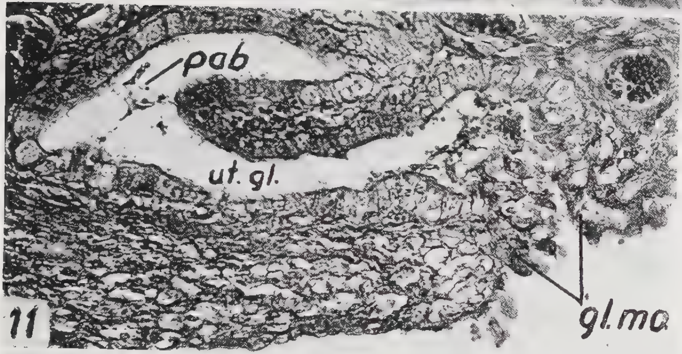
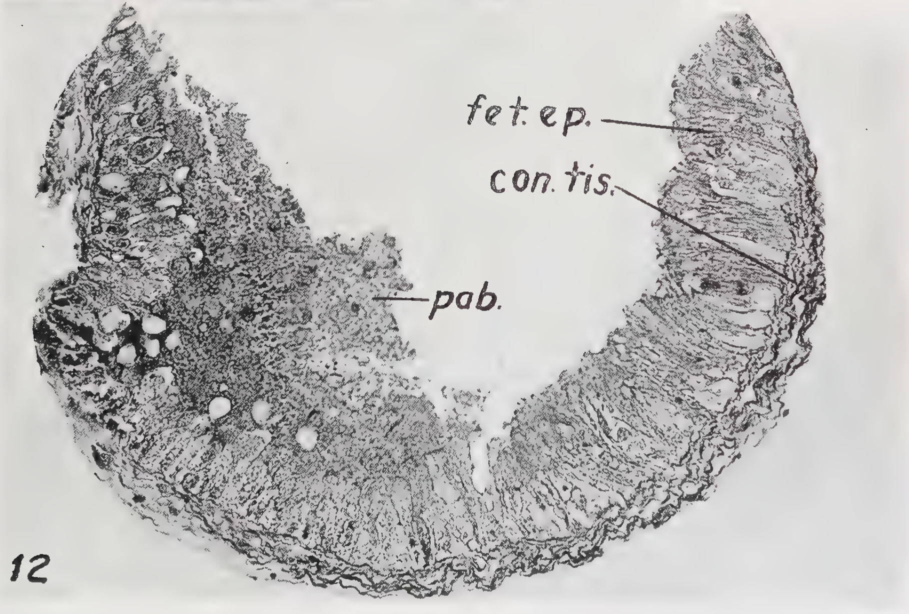

pab
ut. gl.
gl. ma

11


fet. ep.
con. tis.
pab.

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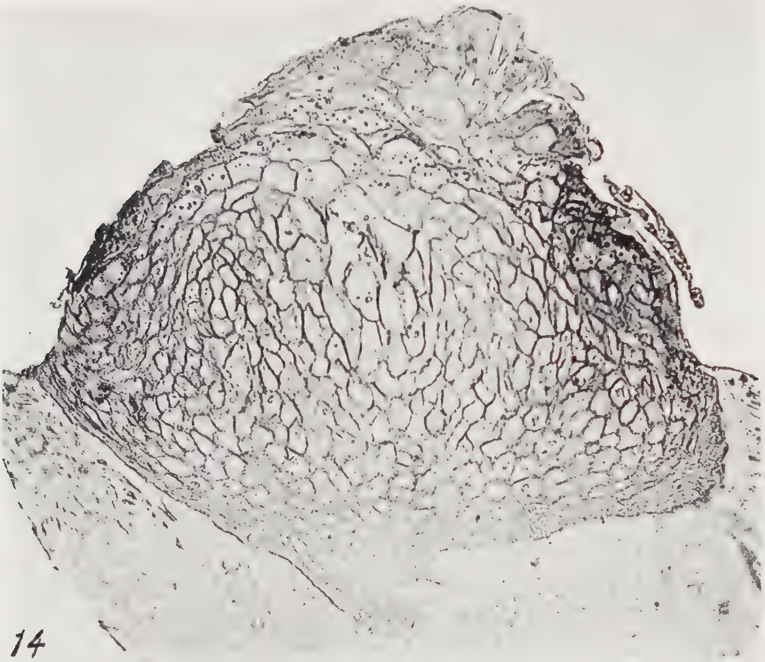

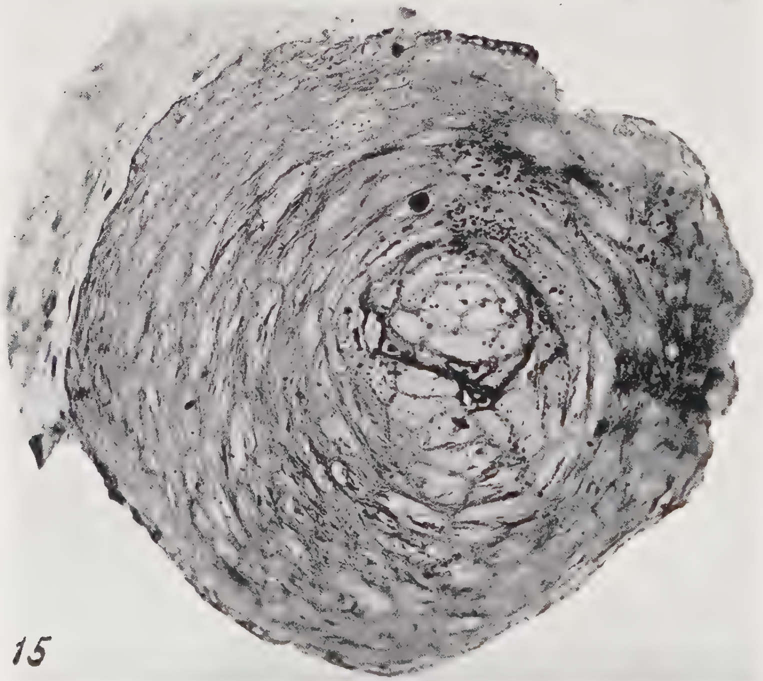

14 Longitudinal section through amniotic cyst. Note small peripheral cells and larger central cells and the dark inclusions contained in the cells of the tip. × 300.

15 Transverse section through amniotic cyst. × 300.

ON THE TERMINATION OF THE CIRCUMFLEX VEINS OF THE THIGH

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ONE FIGURE

Concerning the circumflex veins of the thigh, the relative infrequency of agreement between the text-book description and the condition seen in the cadaver has led to a reexamination of the mode of ending of these blood vessels. Special attention has been given to the comparison of conditions in the white and the American negro. The manner of termination of these veins has been compared also with the origins of the arteries they accompany.

In the text-books of anatomy the medial and lateral femoral circumflex veins are described as terminating in the deep femoral vein—a relation comparable to the origins of the medial and lateral femoral circumflex arteries from the deep femoral artery (Cunningham, Gray, Poirier, and Quain). In both Cunningham and Poirier, however, it is stated that not uncommonly the femoral vein is joined by the medial and lateral circumflex veins. Clear illustrations of the terminations of the medial and lateral femoral circumflex veins are relatively scarce, but in the atlases of Sobotta, Toldt, and Spalteholz the plates show plainly the terminations in the femoral vein. This mode of ending was described by Theile ('43), and by Picqué and Pigache ('09), who report that in the majority of cases studied by them the circumflex veins terminate in the femoral vein. These investigators also found that the ending of the veins is at a level proximal to that of the origin of the corresponding circumflex arteries.

Search of the recent literature has revealed nothing respecting these questions.

The cadavers dissected by students in the Anatomical Laboratory of Washington University, during the years 1926, 1927, and 1929, constitute the material for this study. Records of the dissection were obtained in accordance with the method described by Terry ('29). A total of 165 observations were recorded; of this number, fifty-three were white males, right side; fifty-two white males, left side; and thirty negro males, both right and left sides. At the same time records were obtained also on the origins of the medial and lateral femoral circumflex arteries and these have been used in correlating the types of arteries associated with venous types. A detailed study of arterial types is given by Williams et al. ('30).

The modes of termination of the circumflex veins and of origin of the circumflex arteries found in the present series are shown in the accompanying diagrams (fig. 1); they have been designated as types I, Ia, II, IIa, III, and IV. Type IV includes not only the cases in which the circumflex vessels arise or terminate in the deep femoral vessels, but also certain transitional types between type IV and types I, II, and III; these types are indicated by the Roman numerals V, VI, and VII.

The distribution of the types of venous terminations found on the right side in white males and also the types of arterial origins with which the former are associated are shown in table 1. Considering first the frequency of venous types, one notes the predominance of type I (64.2 per cent) over others; that is, the medial and lateral femoral circumflex veins pour their blood directly into the femoral vein, and do not empty into the deep femoral vein as described so frequently in the text-books. That type Ia (17.0 per cent) is next in frequency is of considerable interest, as this type differs from type I only in that the lateral circumflex system is represented by two veins instead of one. One of the circumflex veins is a tributary of the deep femoral vein in ten cases, the other a tributary of the femoral; in not a single case are both circumflex veins tributaries of the deep femoral vein. Practically the same condition of venous termination holds for the left side in white males (table 2); the exceptions are two cases (3.8 per cent) in which both circumflex veins are tributaries of the deep femoral vein, and one case in which the medial

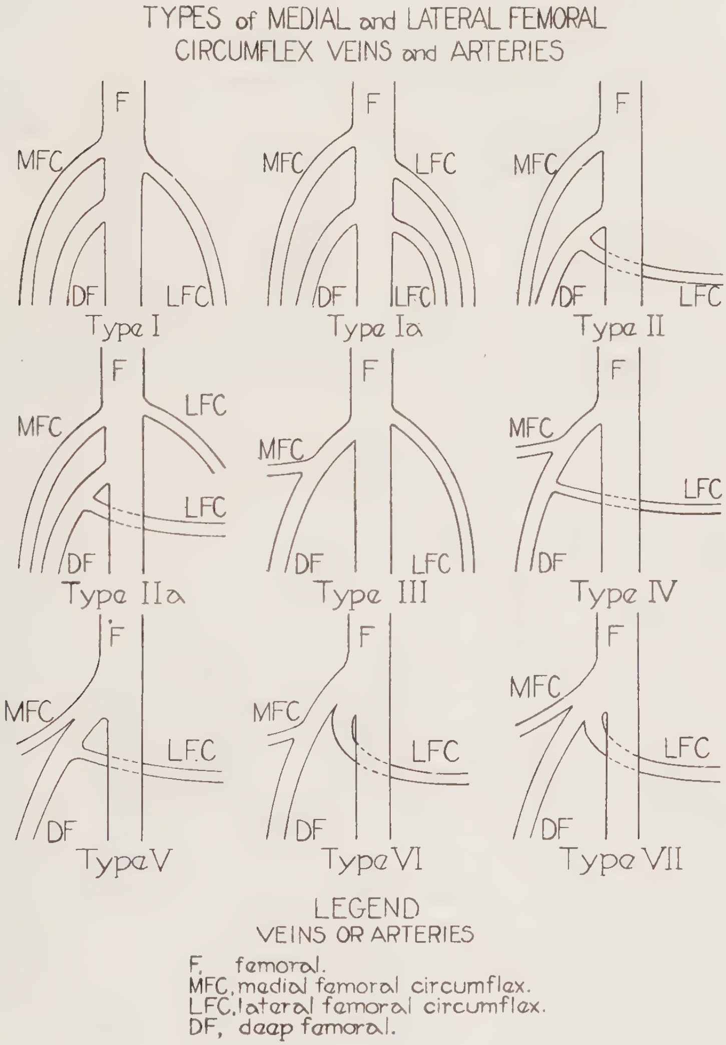
TYPES of MEDIAL and LATERAL FEMORAL CIRCUMFLEX VEINS and ARTERIES
F
MFC
DF
LFC
Type I
F
MFC
LFC
DF
LFC
Type Ia
F
MFC
DF
LFC
Type II
F
MFC
LFC
LFC
DF
Type IIa
F
MFC
DF
LFC
Type III
F
MFC
LFC
DF
Type IV
F
MFC
LFC
DF
Type V
F
MFC
LFC
DF
Type VI
F
MFC
LFC
DF
Type VII
LEGEND
VEINS OR ARTERIES
F, femoral.
MFC, medial femoral circumflex.
LFC, lateral femoral circumflex.
DF, deep femoral.

Figure 1

TABLE 1

Distribution of types of medial and lateral femoral circumflex veins and arteries. White males, right side

VENOUS TYPE	ARTERIAL TYPE					
	I	II	III	IV	Total	Venous type per cent of total
I	2 (5.9)	6 (17.6)	3 (8.8)	23 (67.6)	34	64.2
Ia	1 (11.1)	3 (33.3)	0	5 (55.6)	9	17.0
II	1 (33.3)	1 (33.3)	0	1 (33.3)	3	5.7
IIa	0	2 (100)	0	0	2	3.8
III	1 (20.0)	0	0	4 (80.0)	5	9.4
Total	5	12	3	33	53	
Arterial types per cent of total	9.4	22.6	5.7	62.3		

C = —.451

The figures in parentheses are the percentage of cases in which veins of the specific type (Roman numerals in left vertical column) are associated with arteries of different types (Roman numerals in top horizontal column).

TABLE 2

White males, left side

VENOUS TYPE	ARTERIAL TYPE						
	I	II	IIa	III	IV	Total	Venous type per cent of total
I	2(6.4)	3(9.7)	1(3.2)	3(9.7)	22(71.0)	31	59.6
Ia	0	4(44.4)	0	1(11.1)	4(44.4)	9	17.3
II	0	1(25.0)	0	0	3(75.0)	4	7.7
IIa	0	0	0	0	1(100)	1	1.9
III	0	0	0	0	4(100)	4	7.7
IV	0	0	0	0	2(100)	2	3.8
Unclassified	0	0	0	0	1(100)	1	1.9
Total	2	8	1	4	37	52	
Arterial types per cent of total	3.8	15.4	1.9	7.7	71.55		

C = —.435

circumflex goes into the saphenous vein while the lateral circumflex enters the v. profunda femoris at its termination.

The distribution of venous types in thirty negro males is shown in tables 3 and 4. Type I is most frequent in the negro males on both the right and left sides (70 per cent), with

TABLE 3

Negro males, right side

VENOUS TYPE	ARTERIAL TYPE I	II	IIa	III	IV	Total	Venous type per cent of total
I	2(9.5)	1(4.8)	0	3(14.3)	15(71.4)	21	70.0
Ia	0	0	1(20.0)	1(20.0)	3(60.0)	5	16.7
II	0	0	0	0	0	0	0.0
IIa	0	1(50.0)	0	0	1(50.0)	2	6.7
III	0	0	0	1(50.0)	1(50.0)	2	6.7
Total	2	2	1	5	20	30	
Arterial types per cent of total	6.7	6.7	3.3	16.7	66.7		

C = —.566

TABLE 4

Negro males, left side

VENOUS TYPE	ARTERIAL TYPE I	Ia	II	III	IV	Total	Venous type per cent of total
I	1(4.8)	1(4.8)	2(9.5)	2(9.5)	15(71.4)	21	70.0
Ia	0	0	0	0	4(100)	4	13.3
II	0	0	1(50.0)	0	1(50.0)	2	6.7
IIa	0	0	0	0	0	0	0.0
III	0	0	0	0	2(100)	2	6.7
Unclassified	0	0	1(100)	0	0	1	3.3
Total	1	1	4	2	22	30	
Arterial types per cent of total	3.3	3.3	13.3	6.7	73.3		

C = —.532

type Ia next in frequency. In four cases on the right side and a similar number on the left, one of the circumflex veins is a tributary of the deep femoral, the other a tributary of the femoral. Type-IV venous termination is not present in the negro males. The unclassified case in table 4 is similar to type I, except that the medial femoral circumflex vein terminates in the saphenous instead of in the femoral vein.

By comparing these tables on veins in whites and negroes, it appears that the latter are slightly less variable than the whites respecting the mode of termination of the circumflex veins of the thigh. The per cent of venous type I is greater in the negro than in the white males; there is also a tendency for type Ia to be less frequent in the negro, for type II to be absent, and for type IV not to appear at all.

Practically the same degree of variability is shown on the two sides of the body in the negro males. In whites the left side of the body is slightly more variable than the right, but the difference is probably not great enough to be of any significance.

The frequency of associations of arterial types of origin and venous termination is also shown in the tables. The most frequent arterial type associated with the type-I venous termination is type IV; this condition holds for both white and negro males and on both sides of the body. Type-Ia veins are also associated with type-IV arteries more frequently than with any other type of arterial origin in the present series, except in white males on the left side, where association with type-II arteries is as frequently encountered. The number of cases of other types of venous terminations is so small that their association with types of arterial origins cannot be of any real significance. In general, all types of veins are associated with type-IV arteries.

The coefficients of mean-square contingency are negative in every case, and are sufficiently large to justify a conclusion that there is a medium negative correlation between the manner of termination of the circumflex veins and the origin of the circumflex femoral arteries in both white and negro males. The lowest coefficient is found for the left side of white males (—.44), and the highest for the right side of negro males (—.57).

Although the series of cadavers studied is too small to give very reliable results, some of the conditions present are so striking that it seems probable that the study of a large series would show the presence of similar conditions. The termination of the medial and lateral femoral veins is in the common femoral vein (type I) in the majority of cases in this series, and not in the deep femoral as commonly described in text-books; both the circumflex veins are tributaries of the deep femoral vein in only two cases. The theory that the femoral vein is the terminus of the circumflex veins is strengthened by the fact that type Ia is the next most frequent type, that one in which the blood from the lateral circumflex system is carried by two veins and goes directly into the femoral vein. Our observations, therefore, confirm the earlier description of Theile and the more recent results of Picqué and Pigache. The testimony of the atlases above referred to and the variation pointed out by Cunningham and Poirier are indicative of further observations in support of the facts here noted. As stated above, only tentative conclusions can be drawn from this study and they must be considered only as indications of the conditions that may exist. The method used in this inquiry points out the possibilities of investigation of gross anatomical questions in the routine dissection of the cadaver in a class laboratory.

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THE ORIGINS OF BRANCHES OF THE AXILLARY ARTERY IN WHITES AND IN AMERICAN NEGROES

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The branches of the axillary artery found in the cadaver are confusing to the student because of the great variability from the descriptions and illustrations in text-books and atlases. Analyses of series of dissections have been recorded, but without uniformity of results. Hitzrot[1] ('01) has tabulated in detail his findings on a series of forty-seven arms; DeGaris and Swartley[2] ('28) have made a very comprehensive report based on 512 arms; and Adachi[3] ('28), in his study of the arterial system of the Japanese, included observations on 572 arms.

This report is based on observations made by the authors on dissections of the a. axillaris in 384 arms. It is concerned only with the origin of named branches which are expected to arise directly from the axillary artery. The distribution of the material according to race and sex is as follows: eighty-nine white males, sixty-six negro males, ten white females, twenty-seven negro females.

The usual description of the artery attributes to it six branches: the a. thoracalis suprema, the a. thoraco-acromialis, the a. thoracalis lateralis, the a. subscapularis, the a. circumflexa humeri posterior, and the a. circumflexa humeri anterior. In cases where these six come directly from the axillary artery in the order named the condition has been designated as 'normal.' Variations in origins of any one of these six branches from this plan and also combinations of variations are noted.

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[1] Hitzrot, J. M. 1901. Bull. Johns Hopkins Hosp., vol. 12. pp. 136–145.

[2] DeGaris, Charles F., and Swartley, William B. 1928. Am. Jour. Anat., vol. 41, no. 2, pp. 353–398.

[3] Adachi, Buntaro. 1928. Das Arteriensystem der Japaner. Kyoto.

The supreme thoracic and the thoraco-acromial originated from the axillary in all but five arms; in four of these, which were right arms (two white males, one negro male, and one negro female), the supreme thoracic originated from the thoraco-acromial, and in the fifth, which was a left arm (white male), there was no thoraco-acromial trunk, the various branches of that trunk having direct origin from the axillary.

In axillae where the anterior and posterior circumflex arteries came from a common trunk (ninety cases), this common trunk arose from the subscapular in one axilla of a white male and in one axilla of a negro. When the circumflex arteries arose by a common trunk, the lateral thoracic arose from the subscapular in five white-male axillae (6 per cent), in ten negro-male axillae (11 per cent), in two white-female axillae (2 per cent), and seven negro-female axillae (8 per cent); and from the thoraco-acromial in three white-male axillae (3 per cent), in one negro-male axilla (1 per cent), and in one negro-female axilla (1 per cent).

When the posterior circumflex came from the subscapular (in fifty-one axillae), the lateral thoracic arose from the subscapular also in four white-male axillae (8 per cent), in five negro-male axillae (10 per cent), and in three negro-female axillae (6 per cent); and from the thoraco-acromial in three white-male axillae (6 per cent), in three negro-male axillae (6 per cent), and in one negro-female axilla (2 per cent).

The 'normal' origin of all six branches of the artery most frequently occurred. When this 'text-book' condition was present on one side only, it was more often so on the left than on the right, indicating possibly a greater stability of the pattern on the left side. A greater frequency of variation on the right side is noted in the table, where the percentage of variation of the origins of the branches is seen to be almost always greater on the right than on the left.

TABLE 1

A table indicating the number of arms (left column in each section) and the percentage in which designated conditions are present (right column in each section): on both sides of the same body, on the right side only, and on the left side only

	WHITE MALE, 178 ARMS				NEGRO MALE, 132 ARMS			
	On both sides of same body	On right only	On left only	Total	On both sides of same body	On right only	On left only	Total
'Normal'	56 = 31%	7 = 4%	23 = 13%	86 = 48%	40 = 30%	6 = 5%	16 = 12%	62 = 47%
Anterior and posterior circumflex by common trunk	18 = 10%	15 = 8%	4 = 2%	37 = 20%	14 = 11%	6 = 5%	6 = 5%	26 = 21%
Posterior circumflex from subscapularis	10 = 6%	8 = 4%	5 = 3%	23 = 13%	10 = 8%	5 = 4%	4 = 3%	19 = 15%
Lateral thoracic from subscapularis	18 = 10%	15 = 8%	6 = 3%	39 = 21%	16 = 12%	11 = 8%	5 = 4%	30 = 24%
Lateral thoracic from thoraco-acromial	0	4 = 2%	4 = 2%	8 = 4%	8 = 6%	3 = 2%	1 = 1%	12 = 9%
	WHITE FEMALE, 20 ARMS				NEGRO FEMALE, 54 ARMS			
'Normal'	6 = 30%	3 = 15%	2 = 10%	11 = 55%	8 = 15%	3 = 6%	5 = 9%	16 = 30%
Anterior and posterior circumflex by common trunk	0	1 = 5%	3 = 15%	4 = 20%	14 = 26%	4 = 7%	5 = 9%	23 = 42%
Posterior circumflex from subscapularis	2 = 10%	1 = 5%	0	3 = 15%	0	4 = 7%	2 = 4%	6 = 11%
Lateral thoracic from subscapularis	2 = 10%	1 = 5%	1 = 5%	4 = 20%	14 = 26%	2 = 4%	1 = 2%	17 = 32%
Lateral thoracic from thoraco-acromial	0	0	1 = 5%	1 = 5%	2 = 4%	2 = 4%	1 = 2%	6 = 10%

The origin of the anterior and posterior circumflexes by a common trunk and the origin of the lateral thoracic from the subscapular occurred in almost equal proportion and more frequently than any other variation in all the groups, with the exception of the negro-female group. In this group the origin of the lateral thoracic from the subscapular showed an incidence equal to that of the 'normal' arrangement, while the origin of the anterior and posterior circumflexes by a common trunk exceeded the 'normal' arrangement in incidence. However, no variation occurred frequently enough to justify the suggestion of a different 'normal' or of a second 'normal' arrangement, with the possible exception of the arrangement just pointed out in the negro-female group.

These findings are not wholly in accord with those of Hitzrot, of DeGaris and Swartley, or of Adachi, whose reports were based, likewise, on dissecting-room material. Hitzrot made no mention of either race or sex, nor did he record conditions of the right and left sides separately, "as the only difference found was in the presence or absence of the long thoracic artery this distinction was dropped, the relative difference consisting in the more constant presence of the long thoracic artery on the left side." By the term 'long thoracic artery' we understand its equivalent to the B N A term 'lateral thoracic artery' employed in the present account. DeGaris and Swartley's report indicates no consistent difference in variation of the two sides. There is slight variation, as, for example, the lateral thoracic arises from the thoraco-acromial more often on the left side in the white group and more often on the right side in the negro group, but on the whole, as is noted in their summary, there is a tendency toward symmetry of the two sides. Adachi found no sex difference, but did find a probably significant difference between the two sides of the body, viz., the posterior humeral circumflex originated in conjunction with or from the subscapular more often on the right side than on the left.

In these earlier reports and in the present report the lateral thoracic artery showed probably the greatest amount of variation from what we have considered its 'normal' origin, viz., from the axillary artery. In Hitzrot's group it was absent in forty-three of his forty-seven cases. DeGaris and Swartley found it arising from the thoraco-acromial in more than 60 per cent of their negro series, while in their white series this origin presented itself in about 30 per cent. Adachi found it as a direct branch of the axillary in only 40 per cent of his series, suggesting a variation equal to that of the negro series of DeGaris and Swartley.

No racial difference was found in our male series, the 'normal' arrangement being present in approximately 50 per cent in both races. In the female series, however, this arrangement was present in 55 per cent of the white group (which may not be significant because of the small number of cases) and in only 30 per cent of the negro group—suggesting not only a sexual difference in the entire series, but also a racial difference in the female series. The arrangement which occurred most frequently (43 per cent) in the negro-female series differed from the 'normal' in having the anterior and posterior humeral circumflexes come from the axillary artery by a common trunk. DeGaris and Swartley found the arrangement we have designated as 'normal' in slightly less than 50 per cent of their white series, while the most frequently occurring arrangement (65 per cent) in the negro series presented the lateral thoracic artery arising from the thoraco-acromial.

THE RELATION OF CABOT RINGS TO THE RETICULOFIBRILLAR APPARATUS OF GOLGI

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TWO PLATES (SIXTY-FIVE FIGURES)

INTRODUCTION

The peculiar ring- and loop-shaped bodies characteristic of certain erythrocytes in cases of severe anemia, first described by Cabot ('03), have quite generally been interpreted as nuclear remnants, in particular as persisting nuclear membranes (e.g., Ferrata and Viglioli, '11, and Juspa, '14). Recently, Cesaris Demel ('21) has advanced the view that Cabot rings represent the boundary zone between an inner area of more rarefied hemoglobin and an outer more condensed area. It is the purpose of this investigation to test the validity of Cesaris Demel's interpretation as opposed to the prevailing view, the original interpretation of Cabot.

MATERIAL AND METHODS

The material for the study of Cabot rings consists of smear preparations of the blood of a case of severe anemia, provisionally diagnosed as of the von Jaksch's variety. The preparations were stained with Wright's stain. The case occurred in the Massachusetts General Hospital, and the blood slides were generously given to us in December, 1928, by Dr. W. R. Jordan, then an interne at that institution. The subject was a white male child of eight months. The child was removed from the hospital against advice, with the provisional discharge diagnosis of "Von Jaksch's anemia, myelogenous leukemia, leukemia cutis, eczema, rickets." At the time of removal the leukocytes numbered 56,800; the erythrocytes, 3,500,000. About three weeks later, the child died of pneumonia. No postmortem was done.

The material for the study of the reticulofibrillar apparatus of Golgi consists of red blood corpuscles of man, frog, and salamander (Amblystoma punctatum and Necturus maculosus). The Gamma and Golgi technics were employed separately and in sequential combination, as follows:

Method of Gamma

Wet blood films are treated with a 0.75 per cent potassium-bichromate solution containing 0.5 per cent acetic acid, and one or two drops of a saturated solution of $CuSO_4$, for every 3 cc. of fluid. After five to ten minutes, the films are washed in physiological salt solution, stained with eosin, washed and mounted in distilled water, or dehydrated, cleared, and mounted in balsam.

Method of Golgi

Dry blood films are fixed twenty-four to forty-eight hours in equal parts of saturated solutions of mercuric chloride and potassium bichromate. They are then transferred into equal parts of 2 per cent mercuric chloride and potassium bichromate, to which 5 to 10 cc. of 1 per cent gold chloride and five to ten drops of acetic acid are added to every 50 cc. of the solution. Slides are left in this solution from one to three days and the degree of impregnation of the 'reticulofibrillar apparatus' is regulated accordingly. Wash in distilled water, dehydrate, clear in xylol, and mount in balsam.

Combined methods of Gamma and Golgi

The slides after washing in physiological salt solution following the method of Gamma are transferred directly to the fixative of the method of Golgi.

Additional materials include red blood corpuscles treated according to the neo-Giemsa method of Cesaris Demel ('21), erythrocytes of frog treated with the osmic-acid technic of Ludford ('26) for the demonstration of Golgi substance ('apparato reticulare interno'), erythrocytes of frog subjected to a 1:10,000 solution of Janus green B for the demonstration of mitochondria, certain erythrocytes of salamander with pigment in the perinuclear area, certain senile erythrocytes of the hagfish (Myxine glutinosa) with perinuclear area of condensed hemoglobiniferous cytoplasm, and maturing normoblasts from the blood of a thirteen-day albino rat fetus.

DESCRIPTION

a. Cabot rings

The erythroblast of approximately normal condition, as seen in smears after Wright's stain (fig. 1), contains a relatively large nucleus with close-meshed coarse reticulum. The color contrast between cytoplasm and nucleus is striking; the cytoplasm stains a robin-egg blue, the nucleus a lilac color. A considerable number of these cells may be seen in mitosis (figs. 4 and 5). Approximately normal normoblasts are characterized by a deeper-staining (purple), more homogeneous nucleus (fig. 2) which becomes more compact, smaller, and generally excentric (fig. 3).

Abnormal erythroblasts are, in general, of somewhat larger size, the nucleus appears swollen, and a peripheral area (nuclear membrane), by reason of its deep lilac color, contrasts sharply with the bluish (fig. 6) or purple (fig. 7) nucleus. Overlying the nucleus may appear what seems to be a central projection from the nuclear membrane. This projection is no doubt peripheral to the flattened nucleus (in the smear) and represents a portion of the nuclear membrane, possibly a fold, otherwise secondarily aggregated at the periphery of the circular (flattened) nucleus.

In the normoblast stage abnormality is indicated by relatively great enlargement of the nucleus (figs. 8 to 11). At this stage also the central projection of the nuclear membrane may appear (fig. 12). The nuclear enlargement appears to be the result of dilution of the nucleoplasm, as if by endosmosis, effecting a general diminution in depth of staining reaction. The net result of the process is the production of a relatively large, generally homogeneous nucleus, of lighter blue color, indistinguishable from that of the cytoplasm (fig. 11). This places the persisting nuclear membrane, of deep lilac color, in striking contrast with the bluish cytoplasm. Even in the normoblast of lesser degree of abnormality, the nuclear membrane, by reason of its deeper color, contrasts with the compact purple nucleus (fig. 13). This persisting nuclear membrane represents a Cabot ring of simplest form. Since these rings generally lack a central lattice or even a single radial strand, they are in fact genuine circles. They cannot be interpreted as optical transections of a sphere. It must be assumed, therefore, that the nuclear membrane, originally, of course, completely enveloping the spheroidal nucleus, has become aggregated in one plane by the spreading process involved in making the blood smears. This forces the further assumption that in these cells the nuclear membrane is originally a relatively fluid substance. The uniformly blue color of these early normoblasts indicates a relative sparsity of hemoglobin—another aspect of the abnormal (anemic) character of this blood.

Assuming an original spherical condition of the rings of Cabot, changed into actual circles in the spreading process, the various loop and figure-of-eight forms (figs. 15 to 20) are derivable as the result of the spreading process upon partially collapsed spheres (fig. 14). The figure-of-eight forms would seem to be even more readily derivable from rings (fig. 11) and loops (fig. 12), modified by a secondary twisting process. However, there is no good evidence that the persisting portion of the nuclear membrane is a ring rather than a hollow sphere prior to the flattening of the cells resulting from the making of the blood smear. Moreover, reticulated rings like those of figure 29 give evidence in support of an original condition of the persisting nuclear membrane as the boundary of a sphere.

Figures 21 to 25 illustrate a series of erythroblasts with bilobed nucleus, apparently in process of amitotic division. The special interest in this connection relates to the possible origin of bilobed (figure-of-eight) Cabot rings (fig. 26) from bilobed nuclei. Figure 25 is especially relevant. Here the two lobes of the dividing nucleus are interconnected by two strands. Transformation of the nuclear content in a manner similar to the process in figures 7 to 11, with persistence of the nuclear membrane, would result in a figure-of-eight structure. In cases where the two nuclear lobes are of unequal size (fig. 24) the resulting Cabot ring would have unequal lobes, like that of figure 17.

Figures 26 and 27 are interesting by reason of the sharp contrast in the staining reaction of the cytoplasm. Both contain a figure-of-eight Cabot ring. But the cell of figure 27 stains like the youngest erythroblast; its cytoplasm failed to elaborate any hemoglobin. The pinkish color of the cell, figure 26, indicates the presence of a small amount of hemoglobin.

Figures 28 and 29 show two successive stages in the extrusion of the Cabot ring. This act completes the process of enucleation of the normoblast to produce the erythroplastid. The extracellular Cabot ring of figure 29 is of especial significance. It has the general form of a figure-of-eight, but it clearly consists of more than a single strand. Taken as a whole, it consists of a wide-meshed net. In this case the wall (membrane) of the spheroidal nucleus had not become fully condensed into a ring.

Figures 30 and 31 are significant in that they show a faint nuclear remnant, more or less ring-shaped, after the migration of a more chromatic globule (fig. 30). In figure 31 the migrating globule itself is enveloped by a purple layer, contrasting sharply with the light blue color of the central portion. A similar condition occurs in the case of the small bilobed nuclear globule in the cell, figure 32; and in the more nearly normal cell, with more hemoglobin, of figure 33. The erythroplastids, figures 34 and 35, are added for purposes of comparison as regards both size and staining reaction.

b. Reticulofibrillar apparatus of Golgi

Red blood corpuscles of man, subjected to a special technic devised by Golgi ('19, '20), show a central body approximately coextensive with the biconcavity (figs. 36 to 39). This body appears variously granular or fibrillar, and constitutes the 'reticulofibrillar apparatus,' first described by Golgi for the erythroplastids of man and the normoblasts of guinea-pig. Golgi claims that it is a unique substance, not included among the list of previously described elements in blood cells. It is not identical with the classical Golgi substance ('apparato reticulare interno,' Golgi, '98) as revealed by Ludford's ('26) osmic-acid technic (compare figs. 42 and 50).

The complete body of the reticulofibrillar apparatus of Golgi has an ellipsoidal (fig. 37) or spheroidal (fig. 38) form. When the red corpuscles are subjected to laking for one minute in distilled water, the reticulofibrillar apparatus scatters and moves peripheralward (figs. 40 and 41). This behavior suggests relationship with hemoglobin. To test this surmise the Golgi procedure was superimposed upon cells previously treated with the Gamma technic. The results and interpretations will be given below.

We were interested in finding out whether the nucleated red blood elements of submammalian vertebrates contained a similar reticulofibrillar apparatus. It proved to be extensively present in the erythrocytes of the frog (figs. 42 to 46). The technic outlines very sharply the boundary of the nucleus. The apparatus is apparently condensed on the nuclear membrane. The result is a ring-shaped body. Apparently the nuclear membrane here also became aggregated in the form of a ring in the cells flattened out in the process of making the smears. Seifriz's ('26) microdissection experiments indicate that the area here occupied by the reticulofibrillar apparatus of Golgi is roughly coextensive with a perinuclear area of more concentrated hemoglobin.

Application of the Golgi technic to the erythrocytes of salamander (Amblystoma punctatum) reveals a similar and equally extensive perinuclear reticulofibrillar apparatus (figs. 47 and 48). The erythrocyte of Necturus also contains this apparatus, but in relatively much smaller amount (fig. 49).

Erythrocytes of the frog were studied also with the osmic-acid technic for the demonstration of the Golgi substance (Ludford, '26). The result is shown in figure 50. It agrees with Bhattacharya and Brambell's ('24) figures of Golgi material in the erythrocytes of fowl and turtle. This problematical Golgi substance is likewise segregated in the perinuclear zone, but it is clear from comparisons between figures 42 and 50 that the reticulofibrillar apparatus is a different substance. This same region is occupied also by the mitochondria, as revealed by treatment of the cells with a 1 to 10,000 solution of Janus green B (fig. 51). In certain erythrocytes of the salamander, Triturus viridescens, pigment granules also may occur more or less abundantly in this region. The 'Golgi-substance' as described by Kurashige ('30) for the erythrocytes of the giant salamander of Japan is largely aggregated in the immediate perinuclear region.

Certain senile erythrocytes of the hagfish (Myxine glutinosa) yield suggestive evidence with respect to the perinuclear condensation of hemoglobin (fig. 52). In Wright-stained smears these cells have a narrow perinuclear capsule, of brilliant orange color, in contrast with the light gray color of the outlying cytoplasm. The cytologic evidence shows the segregation of various substances—Golgi bodies (?), mitochondria, pigment granules—as well as a condensation of hemoglobin in the perinuclear zone. It may reasonably be surmised that the reticulofibrillar apparatus of Golgi might represent an artifact related to one or several of these substances. The subsequent combination of the Gamma and Golgi technics was devised to test the hypothesis that the reticulofibrillar apparatus is somehow related to hemoglobin, or more specifically, to relatively richly hemoglobiniferous cytoplasm.

Gamma ('20) first described a peculiar modification of shape in red blood corpuscles of man when subjected to his special technic. The alterations produced include the formation of an outlying membrane ('periglobular ring'), apparently a plasma artifact, and more especially the eruption of a short finger-like process, the 'extraglobular corpuscle' (figs. 53 and 55). In this process the hemoglobin has become largely segregated in a discoid area ('homogeneous zone') immediately beneath the extrusion. The projection itself may become entirely filled with hemoglobin. Subsequent application of the Golgi ('20) technic reveals the reticulofibrillar apparatus largely in the homogeneous (hemoglobiniferous) zone. The evidence seems to demonstrate a very intimate relationship between hemoglobin and reticulofibrillar apparatus.

A remarkable contribution to the question of the significance of the rings of Cabot has recently been made by Cesaris Demel. This investigator interprets these rings as boundary zones between a central more rarefied region and a peripheral region of more concentrated hemoglobin. By application of his neo-Giemsa technic he has apparently demonstrated the coincidence of the periphery of the reticulofibrillar apparatus of Golgi with the boundary zone between the central and peripheral hemoglobiniferous layers, therefore, with the Cabot ring of his interpretation. This work, accordingly, closely associates Cabot rings with reticulofibrillar apparatus. In at least one area of his preparations of normal red blood corpuscles of man, he observed figures, including figure-of-eight forms, very similar to Cabot rings.

We repeated Cesaris Demel's technic. We succeeded in producing ring structures in the red blood corpuscle (fig. 60). We never observed anything as striking as his figure 36, with its figure-of-eight forms. An occasional cell might contain a figure vaguely suggesting a double loop, but the prevailing result was like that shown in figures 61 and 62. These appearances we interpret as artifacts. Occasional chance results simulate Cabot rings.

In this connection conditions in enucleating normoblasts of a thirteen-day rat fetus are very suggestive (figs. 63 to 65). In preparations stained by Wright's method a small blue-staining globule separates from a larger more or less spheroidal purple-staining net. At this stage the cytoplasm stains a deep blue. The chromatic globule eventually leaves the cell. The purple residual body disappears, presumably by intracellular resorption. We interpret the purple reticular remnant as a persisting nuclear membrane. In this condition, especially when more sharply aggregated in ring form, it suggests a Cabot ring. Persistence of such nuclear remnant (nuclear membrane) in certain normoblasts of the blood in cases of severe anemia would seem to explain the origin of Cabot rings.

DISCUSSION

The reason for associating the rings of Cabot ('03) and the reticulofibrillar apparatus of Golgi ('20) in the same investigation arises from Cesaris Demel's claim in a recent paper ('21) in which he derives Cabot rings from the peripheral portion of the Golgi apparatus. Since in erythrocytes the perinuclear region is the locus also of segregation of mitochondria, alleged Golgi substance, pigment, and hemoglobin, a consideration of the significance of the reticulofibrillar apparatus of Golgi involves the question of a possible genetic relationship between one or several of these cytoplasmic elements and the Golgi apparatus. Cesaris Demel interprets Cabot rings in terms of a more deeply staining (with Giemsa and neo-Giemsa technics) border between an inner zone with rarefied hemoglobin and a peripheral zone of denser consistency.

To avoid confusion, we will use the term Golgi apparatus to indicate the 'reticulofibrillar apparatus' of Golgi, first described in 1919 and interpreted as a unique cytoplasmic constituent, unrelated to any previously revealed cytoplasmic element. The term Golgi substance we will use to refer to the classical 'apparato reticulare interno' described by Golgi in 1898 in cells treated by silver technics.

With reference to Cesaris Demel's interpretation of Cabot rings, it may be stated first that, contrary to his opinion, the evidence indicates a relatively greater accumulation of hemoglobin in the perinuclear area as compared with the outlying cytoplasm. This reversed interpretation as regards relative local accumulation of hemoglobin nevertheless leaves a similar more deeply staining dividing zone, and allows practically the same basis for Cesaris Demel's interpretation of Cabot rings.

That hemoglobin is relatively more concentrated in the perinuclear region, at least in certain cells, is strikingly suggested in the case of certain senile erythrocytes of the hagfish (fig. 52). In Wright-stained smear preparations these cells show a thin layer of brilliant orange color immediately enveloping the dissolving nucleus. If the nuclear membrane suffered complete resorption with the inner nucleoplasm, which apparently is not the case, then the peripheral cytoplasmic zone of condensed hemoglobin might provide the basis for the formation of a Cabot ring. The findings of Seifriz ('26) in connection with his microdissection studies of the erythrocyte of certain amphibians (Cryptobranchus; Amphiuma) show that the hemoglobin-containing substance reaches its maximum thickness in the perinuclear region.

We may consider first the direct evidence in support of the original (Cabot, '03) and prevailing view regarding the derivation of Cabot rings. The strictly ring form (fig. 11) can readily be traced back directly to the nuclear membrane (fig. 7). Beginning with the basophilic erythroblast stage (fig. 6) in cases of severe anemia, the nuclear membrane contrasts sharply as a deep violet ring with the lighter bluish nucleoplasm. This ring, the nuclear membrane, persists through successive stages, while the nucleoplasm becomes progressively more diluted and in consequence homogeneous (figs. 8 to 12). In such normoblasts the cytoplasm remains orthobasophilic or at most becomes slightly polychromatophilic (figs. 10, 12, 17, 26).

A peculiarity of the nuclear membrane and the ensuing Cabot ring in certain cells is significant. The chief difficulty in interpreting Cabot rings in terms of nuclear membranes inheres in the fact that, while the nuclear membrane must bound a sphere, in consequence must constitute an originally spheroidal structure, the Cabot ring is a circular body. The original Cabot ring is not an optical section of a sphere. This fact forces the assumption that in the flattening process of making blood smears the complete membrane must segregate into an annular structure; which must rest on another assumption, namely, that the nuclear membrane is semifluid and very labile. These assumptions are supported by the peculiarity above specified. This consists of one or several connecting strands or projections (figs. 6, 7, 12, perhaps 15, 16, and 20). The strongest evidence is contributed by such Cabot rings as that illustrated in figure 29, where the extruded ring consists of a reticulated structure. It is uncertain whether rings like those of figures 15 and 16 are properly interpretable as rings with central strands or rings in process of twisting into figure-of-eight loops (figs. 17, 18, 19, and 26). These figure-of-eight forms are readily derivable from collapsing rings (fig. 14) further modified by a spiral twist, the result of the operation of mechanical factors incidental to liquefaction of the nucleoplasm and subsequent exosmosis. The fact that Cabot rings may ultimately be extruded from the normoblast (figs. 28 and 29) is further evidence, in view of the normal extrusion of the nucleus, in favor of their nuclear derivation.

Possibly the best evidence for the nuclear-membrane origin of Cabot rings is afforded by enucleating polychromatic normoblasts, like those of figures 30 and 31. These are especially significant, in view of the normal manner of enucleation of normoblast of the rat fetus (figs. 63 to 65). In figure 30 a compact chromatic globule has migrated to the periphery of the cell, presumably anticipatory to extrusion. Centrally a faint violet ring is discernible, of approximately the size of the original nucleus. This is obviously the homologue of the more typical Cabot ring. It apparently represents a more nearly normal condition, one in which the remnant of the nuclear membrane also suffers resorption or extrusion.

Figure 31 illustrates a similar condition, except that the migrating chromatic globule is itself enveloped by a violet-colored ring, apparently the precursor of a Cabot ring of relatively small size. In the normoblasts, figures 32 and 33, the same phenomenon of the development of small Cabot rings from small contracted nuclei or nuclear fragments is illustrated. The fact that small rings, similar in every respect except size to typical rings, may arise from nuclear fragments further supports the view of a nuclear-membrane origin of Cabot rings.

In the normoblasts from a thirteen-day albino rat fetus the normal method of enucleation involves the separation of a globule of nucleoplasm from a larger sharply contoured reticular structure, and its peripheral migration and subsequent extrusion. The central body apparently represents the nuclear membrane. In Wright-stained preparations it has the same violet color as the typical Cabot ring. Condensed peripherally, as in figures 63 and 65, it resembles closely a Cabot ring and most probably has the same significance. Subsequently it disappears either by intracellular resorption, as in figures 30 and 31; or by extrusion, as in figures 28 and 29.

The occurrence of numerous normoblasts with bilobed nuclei suggests that the bilobed (figure-of-eight) Cabot rings may originate from this source rather than by process of collapse and spiral twisting of annular bodies. Evidence in favor of the latter mode of origin seems too clear (figs. 14, 16, and 17) to ignore. However, such evidence as that furnished by figure 25, where the two lobes of the nucleus are interconnected only by two delicate purple strands, in color and girth like the material of Cabot rings, strongly suggests origin of certain bilobed Cabot rings from bilobed nuclei.

On the other hand, the series of stages, figures 21 to 25, suggest successive early steps in amitosis. That this type of erythroblast and normoblast proliferation occurs cannot be definitely disproved. The occurrence of mitotic divisions of erythroblasts (figs. 4 and 5), however, might be thought to render doubtful concomitant amitosis. Possibly, of course, both modes of erythroblast multiplications might occur. This is true of the blood of certain lower forms (e.g., lung fish and salamander, Jordan and Speidel, '30).

The undoubted occurrence of mitotic division of erythroblasts in the peripheral blood of this case of severe anemia seems of much theoretical interest. In the blood of fishes and amphibians (Jordan and Speidel, '30) this phenomenon is normal and of considerable frequency. Under normal conditions in mammals the erythroblasts, and other ancestral blood cells, proliferate in the central hemocytopoietic tissues, chiefly bone marrow. Under the abnormal conditions of myeloid dyscrasias associated with certain types of severe anemia, the precursors of the erythrocytes may proliferate in the peripheral blood stream, as normally in lower forms.

Cesaris Demel ('21), by application of special technics, disclosed in red blood corpuscles a central network, from the periphery of which, through condensation, he derives Cabot rings. This central network is believed to be coextensive with a central area of relatively more rarefied hemoglobiniferous cytoplasm. It colors reddish with Giemsa and neo-Giemsa stains. In structure, position, and extent it seems identical with the so-called reticulofibrillar apparatus of Golgi ('20). With the Golgi technic it stains dark brown or black. Cesaris Demel suggests that these central reticular bodies are the same. Proceeding on this basis, the first step in an effort to check Cesaris Demel's interpretation of Cabot rings is to seek the significance of the reticulofibrillar apparatus of Golgi.

That the perinuclear region in erythrocytes is relatively richer in hemoglobin is indicated by the microdissection studies of Seifriz ('26), and by conditions in certain senile red cells of Myxine (fig. 52). This is exactly the region to which the reticulofibrillar apparatus of Golgi is limited (figs. 42 to 49). This is the region also of segregation of Golgi substance (fig. 50), mitochondria (fig. 51), and pigment (in certain erythrocytes of salamander). But these materials are too small in amount to account for the much more extensive reticulofibrillar apparatus of Golgi. They may accordingly be disregarded, assuming that they could persist after the Golgi technic, in a consideration of the origin of the reticulofibrillar apparatus of Golgi.

On the hypothesis that the Golgi apparatus might be related to the relatively condensed hemoglobin of the perinuclear area, we attempted to shift the distribution of the hemoglobin with the view of producing a corresponding shift of the reticulofibrillar body. Such shift of hemoglobin was possible by means of laking the cells in distilled water, or by subjecting them to the technic of Gamma ('20). In the case of anucleate red corpuscles the assumption seems logical that following loss of nucleus the entire central area, that is, the area roughly coextensive with the biconcavity, of the red blood corpuscles of man becomes the region of greater condensation of hemoglobin. This is the region of the reticulofibrillar apparatus of Golgi (figs. 36 to 39). When the red corpuscles were laked for one minute in distilled water and then subjected to the Golgi technic, the reticulofibrillar apparatus appeared peripherally (figs. 40 and 41).

The Gamma technic gives even more striking and positive evidence of a close relationship between hemoglobin and Golgi apparatus. This technic effects a polar aggregation of the hemoglobin into a short finger-like protrusion and an underlying discoid area (figs. 53 and 55). Subsequent application of the Golgi technic discloses the reticulofibrillar apparatus largely in the region of hemoglobin segregation. Exactly the same results follow in the case of the erythrocytes of the frog (figs. 57 to 59). The evidence seems complete that the reticulofibrillar apparatus is somehow related to hemoglobin. The suggestion presents itself that it may be of the nature of a precipitation or coagulation artifact with richly hemoglobiniferous cytoplasm.

That the result of subjecting the cells to this technic is not simply one of independent shift of hemoglobin and a preformed Golgi apparatus in the form of a net, to the same regions under the influence of the same physicochemical factors, seems obvious. The presence of the nucleus in the erythrocytes of frog would seem to be a sufficient anchor for an actual perinuclear network to prevent its being moved completely to the polar region (fig. 59).

Since Cabot rings arise in normoblasts with little or no hemoglobin (figs. 7 to 20), and since the reticulofibrillar apparatus seems definitely related to hemoglobin, there would appear to be no possibility of genetic relationship between the two. Moreover, the Cabot rings seem clearly to represent persistent nuclear membranes, while the reticulofibrillar apparatus of Golgi represents a perinuclear substance.

The conclusion that Cabot rings have no relation to the reticulofibrillar apparatus, however, imposes the necessity of a reasonable explanation of the ring and figure-of-eight bodies described by Cesaris Demel as derived from the central fibrillar body revealed by his technic. We have repeated his technic, with results that we can only interpret as artifacts (figs. 60 to 62). To be sure, numerous ring forms occur, in every way comparable to those discussed by Cesaris Demel. But we have been able to observe no clear-cut figure-of-eight forms. Assuming, nevertheless, that they occur as described by Cesaris Demel, after expert application of his technic, they need not be interpreted as true Cabot rings. They are mere simulacra of true rings. If they arise, as described by Cesaris Demel ('21), by peripheral condensation of the central fibrillar body (whatever the true relation to hemoglobin), as annular elements they might very readily be subject to the same mechanical factors that convert a ring-shaped remnant of nuclear membrane into a figure-of-eight body, as in the case of authentic Cabot rings.

The findings of Brodersen ('15) in certain cartilage cells substantiate this view. These cells, apparently in process of fatty degeneration, contain variable numbers of extranuclear rings and loops, including figure-of-eight forms resembling Cabot rings. Given a cytoplasmic globule of fatty or other substance, a structure presents itself very similar to the mass of nucleoplasm of certain anemic normoblasts, which under the effect of similar physicochemical factors would suffer similar morphologic alterations. Such alterations, as in the case of Cesaris Demel's treatment of red blood corpuscles and Brodersen's treatment of cartilage cells, could readily result in the production of close simulacra of genuine Cabot rings.

The local hemoglobiniferous protrusion resulting from the application of the Gamma technic calls for explanation. Why has it the form of a finger-like projection, and why is there only one protrusion (figs. 53 to 56)? Obviously there is a local area of relative elasticity or weakness. Otherwise one would expect protrusions of variable size and shape, including especially dome-shaped projections. Release of tension through local extrusion would restore internal equilibrium, and account for only one rather than several eruptions. The further question then arises whether the protrusion is always at the same point. This defies satisfactory answer. Assuming that there is one special point in the plasma membrane of relatively less resistance, then one might suggest that this point represents the final area of separation of the daughter cells following mitotic division.

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PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 35 are cells from smear preparations of blood from a case of von Jaksch's anemia. Wright's stain. Magnification, 1350 diameters. Drawings of figures 1 to 35 by J. E. Kindred; figures 36 to 65 by Alice Clarke Mullen.

1 and 2 Erythroblasts.

3 Normoblast.

4 and 5 Erythroblasts in mitosis.

6 and 7 Normoblasts, with swollen nucleus and contrasting nuclear membrane.

8 to 11 Normoblasts, in which the swollen nucleus has become practically homogeneous. The nuclear content in this stage takes a blue stain, thus causing the purple-staining membrane to contrast sharply.

12 Normoblast in which, as occurs less conspicuously in figures 6 and 7, the nuclear membrane shows a superficial strand.

13 More nearly normal normoblast, as concerns both hemoglobin content and contrasting character of the nuclear membrane.

14 Persistent nuclear membrane (Cabot ring) partially collapsed.

15 Partial twisting of collapsing Cabot ring.

16 Further twisting of collapsing Cabot ring.

17 Cabot ring completely twisted into a figure-of-eight shape.

18 and 19 Figure-of-eight-shaped Cabot rings, bent into semilunar forms, in normoblasts practically lacking hemoglobin.

20 Odd form of Cabot ring.

21 to 25 Normoblasts with bilobed nucleus. The persistent nuclear membrane would have an essentially figure-of-eight shape.

26 Bilobed (figure-of-eight) Cabot ring, possibly representing the persistent nuclear membrane of a normoblast with bilobed nucleus, e.g., figure 24. The pink-staining reaction of the cytoplasm indicates a moderate amount of hemoglobin.

27 Bilobed Cabot ring, in basophilic normoblast.

28 and 29 Two successive stages in the extrusion of the Cabot ring. The reticular character of the extruded ring is more suggestive of a complete nuclear membrane.

30 Normoblast (basophilic) showing a small peripheral chromatic body apparently in process of extrusion, and a more central larger ring. The latter presumably represents the persistent nuclear membrane (Cabot ring).

31 Normoblast, similar to that of figure 30, but containing a moderate amount of hemoglobin. The migrating spheroidal mass of chromatin is itself enveloped by a purple membrane, resembling a small Cabot ring.

32 More nearly normal normoblast with small bilobed nucleus, the sharply contrasting membrane of which presumably might persist as a small bilobed Cabot ring.

33 Nearly normal normoblast, the condensed nucleus of which has suffered a considerable amount of resorption, leaving a small Cabot ring.

34 and 35 Two normal red blood corpuscles, for comparison as regards size and staining reaction, with the normoblasts containing Cabot rings.

RINGS OF CABOT
H. E. JORDAN, J. E. KINDRED, AND H. W. BEAMS

PLATE 1

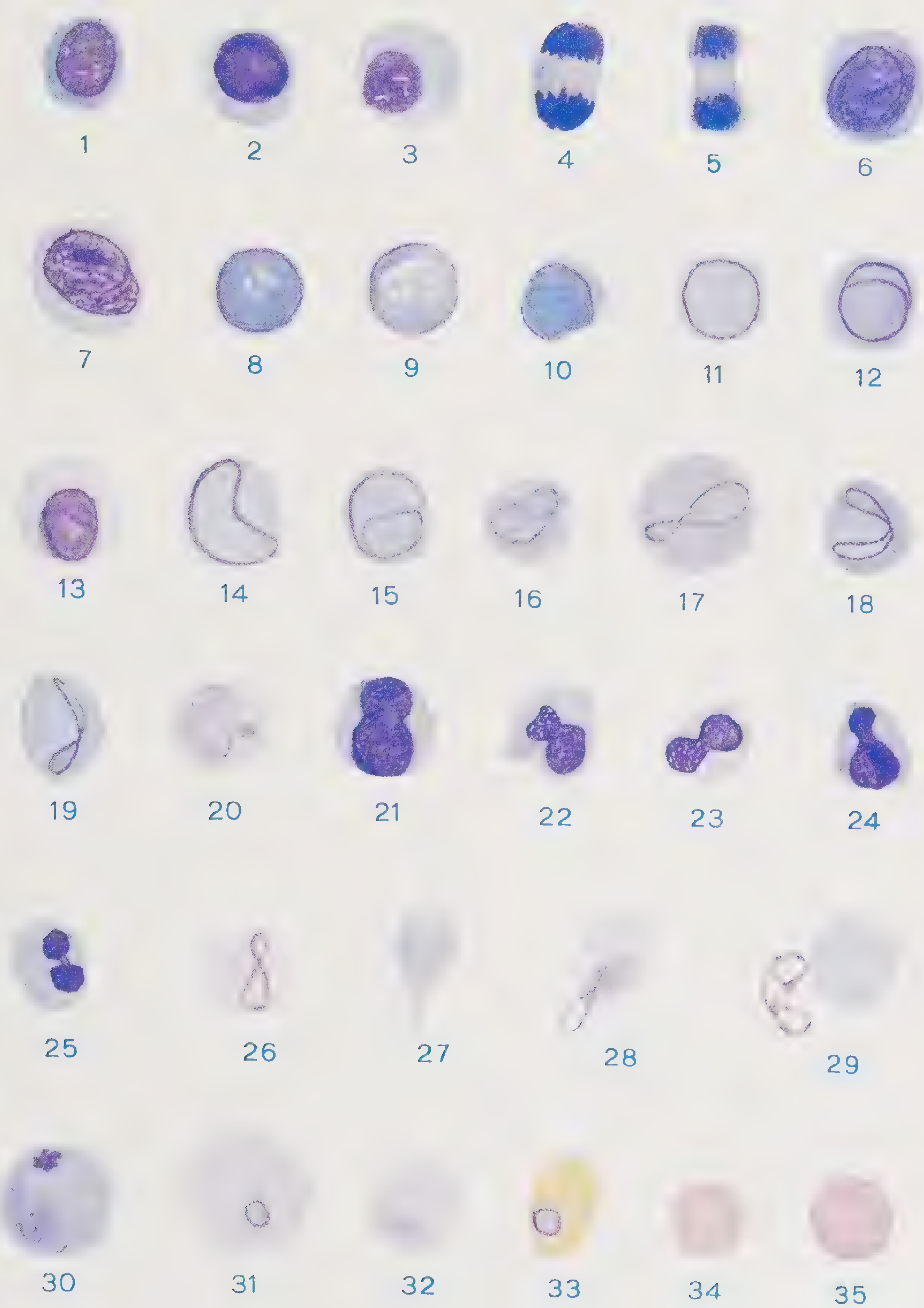
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PLATE 2

EXPLANATION OF FIGURES

36 to 39 Normal red blood corpuscles of man, showing reticulofibrillar apparatus of Golgi. Golgi ('20) gold-chloride technic. Magnification, 1350 diameters.

40 and 41 Red blood corpuscles of man, after laking for one minute in distilled water. The scattered reticulofibrillar apparatus appears peripherally. Golgi ('20) technic. × 1350.

42 to 46 Erythrocytes of frog. The ellipsoidal nucleus appears centrally, enveloped by the reticulofibrillar apparatus. Golgi ('20) technic. × 1350.

47 and 48 Erythrocytes of salamander (Amblystoma punctatum), showing the reticulofibrillar apparatus of Golgi. × 1350.

49 Erythrocyte of Necturus. Here the reticulofibrillar apparatus of Golgi occupies a relatively less wide perinuclear area. At the upper left-hand border occur several globules of the segregation apparatus. × 1350.

50 Erythrocyte of frog, treated according to the osmic-acid technic of Ludford ('26) for disclosing the classical Golgi apparatus ('apparato reticulare interno'). The Golgi substance (?) occurs in the form of globules of varying size in the perinuclear area. × 1350.

51 Erythrocyte of frog, treated with a 1: 10,000 solution of Janus green B to reveal the mitochondria. These elements occur as granules and short rodlets also aggregated in a narrow region surrounding the nucleus. × 1350.

52 Senile erythrocyte of the hagfish (Myxine glutinosa). A narrow, apparently more condensed layer of cytoplasm envelops the liquefying nucleus. In preparations treated with Wright's stain, this envelope takes on a brilliant orange color, in sharp contrast with lighter gray color of the remaining cytoplasmic area. The staining reaction suggests aggregation of the hemoglobin. × 1350.

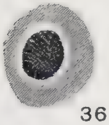
53 Red blood corpuscles of man, treated according to the technic of Gamma ('20). This treatment produces a finger-like projection ('corpicciolo extraglobulare') at one point. The hemoglobin becomes largely segregated in a discoid area ('zona homogenea') immediately beneath the projection. Central to this occurs a granular crescentic area ('zona granulosa') (fig. 54). At a variable distance from the corpuscle appears a delicate membrane ('anello periglobulare,' figs. 54 and 56), apparently a coagulation product of the plasma. × 1350.

54 Red blood corpuscle of man treated according to the Golgi ('20) technic following the Gamma technic. The reticulofibrillar apparatus of Golgi is largely segregated in the hemoglobin-containing area, the 'zona homogenea.' × 1350.

55 Red blood corpuscle of man, modified by the Gamma technic. The hemoglobin completely fills the extraglobular corpuscle and is continuous with that of the homogeneous zone. × 1350.

56 Red blood corpuscle of man, treated with the Golgi ('20) technic following the Gamma technic. Here the reticulofibrillar apparatus of Golgi appears both in the extraglobular corpuscle and in the zona homogenea. The periglobular ring is sharply defined. × 1350.

(*Continued on page 160*)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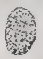
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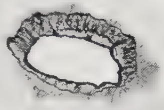
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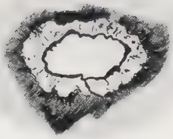
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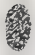
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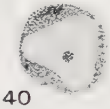
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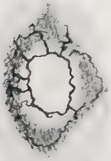
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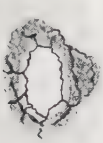
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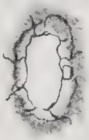
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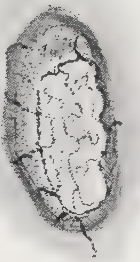
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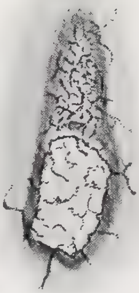
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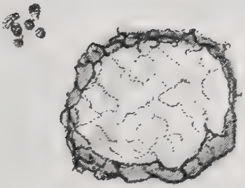
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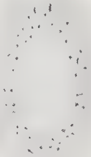
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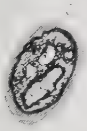
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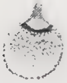
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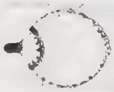
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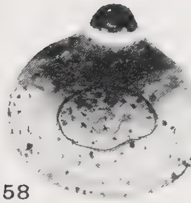
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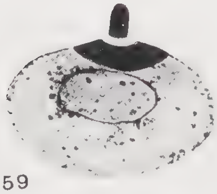
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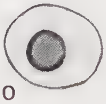
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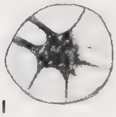
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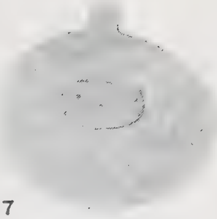
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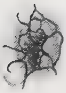
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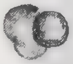
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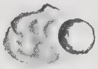
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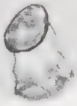
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57 Erythrocyte of frog, after the Gamma technic. The nucleus is faintly outlined in the center. Between the nucleus and the polar projection ('corpicciolo extraglobulare') appears distinctly the discoid 'zona homogenea' in which the hemoglobin is largely segregated. × 1350.

58 and 59 Two erythrocytes of frog, treated with the Golgi ('20) technic following the Gamma technic. The reticulofibrillar apparatus of Golgi is seen to have been segregated in the projection (extraglobular corpuscle) and the subjacent homogeneous zone, i.e., in the areas of hemoglobin segregation. × 1350.

60 to 62 Three general varieties of appearance of the red blood corpuscles of man following the application of Cesaris Demel's ('21) neo-Giemsa technic, for the study of Cabot rings. Figure 60 represents a more successful (?) result, and according to Cesaris Demel, the central ring-shaped body is comparable to a Cabot ring. The various types of rings and networks are, in our opinion, interpretable as artifacts.

63 to 65 Three normoblasts drawn from smear preparations of blood from a thirteen-day albino rat fetus. These three normoblasts show successive early stages in the enucleation process. A more chromatic (blue after Wright's stain) homogeneous portion of the nucleus separates from a larger more reticular portion (purple after Wright's stain). The blue globule is eventually extruded, while the purple network seems to disappear, following a ring stage, by intracellular resorption. The ring stage of the nuclear remnant (nuclear membrane) is apparently the homologue of the Cabot ring of anemic red blood corpuscles. × 1350.

CHANGES IN THE ERYTHROCYTES OF NECTURUS ASSOCIATED WITH THE INTRACELLULAR CRYSTALLIZATION OF HEMOGLOBIN

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ONE PLATE (TWELVE FIGURES)

The phenomenon of intracellular crystallization of hemoglobin in the erythrocytes of Necturus is apparently well known to both biochemists and physiologists. Stewart ('18, p. 71) describes it as occurring regularly after exposure of the blood of this animal to either saponin or sodium taurocholate, and Mathews ('21, p. 498) makes the following statement: "Moreover, when hemoglobin is set free in the corpuscle by some of these methods (laking), particularly in the very large cells of Necturus, a tailed amphibian, the hemoglobin may crystallize in the corpuscle itself."

This phenomenon was first observed by the writer while studying blood from animals which had been exposed for varying periods to a dilute solution of lead acetate, and the readiness with which such intracellular crystallization could be induced prompted the present study of the associated changes occurring within the erythrocyte.

MATERIAL AND METHODS

The blood was obtained from Necturus which had been immersed for varying periods in a dilute solution of lead acetate (1:20,000). In these animals definite evidence of injury of erythrocytes was obtained as early as the fifth day of exposure and this was accompanied by an active phagocytosis of the modified erythrocytes within the blood vessels

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(Dawson, '30 b). The formation of hemoglobin crystals within the red blood cells was induced by the momentary drying of drops of blood on the slide before covering. Only the peripheral blood cells in contact with the glass slides appeared to be so affected and, in a covered preparation, they were accordingly found distributed in a circular pattern, outlining the original limits of the drop. Occasionally a few cells were dislodged, but the majority were sufficiently dried to the glass to resist the peripheral rush of blood cells which occurred when the cover-slip was applied. Crystallization is not entirely confined to the blood of lead-poisoned animals, but may occur in rare instances in the blood of apparently normal animals.

Preparations made in this manner were subsequently sealed with warm vaselin to permit detailed study. Clean slides and slides filmed with both neutral red and neutral red in combination with Janus green B were used. In some cases additional neutral red was applied to the preparation by replacing the first cover-slip with a second which had been also filmed with dye. Permanent preparations of supravitally stained blood were made by the method of Scott ('28).

DESCRIPTION

On account of the rapidity with which the crystals form, together with the fact that no further crystallization of hemoglobin has been observed after the preparation is covered and sealed, it is difficult to gain any definite idea of the appearance of the cells in the early stages of crystal formation. However, by varying the time of exposure to the air and making a sufficient number of preparations, a variety of stages can be secured, but there are none that are clearly associated with the initial stages of the process.

In preparations secured by the methods outlined it is found that more or less of the hemoglobin within the cell may be crystallized. Undoubtedly the reaction would be completed if it were not halted by covering and sealing the blood. In some instances (figs. 1 and 2) the cells do not appear greatly modified or distorted. The segregation apparatus is often typical and the mitochondria have the form and distribution characteristic of fully matured erythrocytes (Dawson, '30 a). The single crystal is surrounded by apparently normal cytoplasm, but the hemoglobin concentration is reduced. Frequently in the vicinity of the crystal the wall of the corpuscle is indented as if it were influenced by some attraction to the crystal.

In other cases of partial crystallization (figs. 3 and 4) the long axis of the crystal does not coincide with the long diameter of the erythrocyte and the corpuscle accordingly suffers more or less distortion. The mitochondria, however, usually remain grouped in the vicinity of the nucleus, but the granules of the segregation apparatus are frequently greatly displaced, and in some instances both these elements and the mitochondria in the vicinity may even appear to be incorporated within a crystal.

In erythrocytes in which crystallization of hemoglobin is complete, the size and number of the crystals vary greatly. It is difficult to determine whether this is due to initial variations in the amount of hemoglobin in individual cells or whether hemoglobin is to some extent lost from certain cells due to injury brought about by the method of inducing intracellular crystallization. In such a cell as shown in figure 5 crystallization is complete, that is, no visible hemoglobin is present other than that in the crystal, but the crystal is very small when compared with those of other cells (figs. 6 to 12) In this particular cell the mitochondria were clearly visible without being stained and some appeared to be oriented with reference to the crystal rather than to the surface of the nucleus. A greatly distorted cell is shown in figure 6. This was stained supravitally with neutral red-Janus green B and the distribution of mitochondria and neutral-red granules was clearly observed.

The other cells shown in the plate illustrate chiefly variations in the size, number, shape, and arrangement of the crystals within the erythrocytes. In all such corpuscles the cell membranes become conspicuous, appear to be real structures, and are frequently deformed by the projecting ends of especially long crystals (fig. 10). The membranes are also usually indented or indrawn toward the surface of large crystals, the point of greatest concavity of the membrane being about midway of the long axes of the crystals.

Description of the crystals seems unnecessary, since they have been figured and described by Reichert and Brown ('09) in their monograph on the crystallography of hemoglobins. The crystals within the cells resembled those formed from laked blood, only they were much smaller. Within the cell, the nucleus occasionally modifies the form of the crystal or causes it to be imperfect. The irregular crystals shown in some figures were outlined twenty-four hours after the crystals were first formed, and doubtless some of the atypical structures represented are due to this fact.

Crystal formation is also associated with a greatly decreased viscosity of the cell contents. This is indicated by the increased brownian activity of the neutral-red granules and is also clearly demonstrated when the cells are manipulated by varying the pressure on the cover-slip. The crystals and even the nucleus are then observed to slip freely about the cell, but they tend to return to their original position when the pressure is withdrawn.

An effort was also made to determine if reticulation could be induced in these modified cells (Dawson, '29). The original cover-slip which was not filmed with neutral red was removed and replaced by a second one which carried a film of the dye. Ordinarily an increase in the concentration of the dye will favor the induction of secondary neutral-red granules and frequently will result in the development of reticular patterns. In these experiments, however, the modified cells did not exhibit any additional reaction to the dye, while the apparently normal cells responded in a typical manner.

DISCUSSION

In such as this, admittedly a description only of the morphological aspects of the phenomenon of intracellular crystallization, it seems unwise to attempt without more experimental evidence any prolonged discussion of the factors involved in maintaining the hemoglobin within the red blood cell in solution.

It has long been recognized that different animals' hemoglobins differ in solubility and ease of crystallization, and, as Schäfer ('29) points out, hemoglobin rarely crystallizes within the corpuscles. He makes the suggestion, apparently based on certain observations of Adrian, that the salts the corpuscles contain keep the hemoglobin in solution. On the other hand, Barcroft ('28) states that the red blood corpuscles of many animals, if hemolyzed even under the most rigorous conditions and without either concentration or dilution of the hemoglobin, shed it as crystalline material. He suggests that within the cell the molecules are not free to orientate themselves at pleasure, and that the direction of their orientation is in some way decided by the structure of the cell and the whole of the biological aspect is regulated by that orientation; or, as he infers elsewhere in the same volume, certain properties of hemoglobin are not merely due to its chemical composition, they are due in part to the fact of its being housed in corpuscles.

A somewhat similar idea is also expressed by Seifriz ('26) after observing the behavior of the erythrocytes of Cryptobranchus when torn by the needles in microdissection. He found that the loss of the hemoglobin was not through the tear, but over the entire surface of the wall, due, in part, to general increased permeability. He believed, however, that another factor was involved, disorganization of the cytoplasm. Without this latter condition, he points out, the loss of hemoglobin would not be possible, that is, if we are to regard the contents of the corpuscle as a dilute jelly with continuity of structure rather than a solution of hemoglobin.

Guerber ('27) has also observed intracellular crystallization of hemoglobin in the nucleated erythroblasts of embryos of the pig and the cow, but failed to find it in similar cells of the guinea-pig, dog, and man. In the case of the erythroblasts from the pig and cow the crystals of hemoglobin could be returned to solution by a slight increase in temperature, but this did not occur when the blood was laked and the crystals had formed outside the cells. He concludes that it is possible that this crystallization involves a reversible intermingling of the colloidal system of the cytoplasm of the erythroblast and that the hemoglobin is not deutoplasm, but is a protoplasmic constituent of the erythrocyte.

In the case of intracellular crystallization in Necturus there is an evident loss of viscosity of the protoplasmic contents, but no visible evidence of the loss of any constituent through the cell membrane. Contact with the glass slide on the periphery of the drop and slight drying appear to be the only differential factors involved in inducing the formation of crystals of hemoglobin. Many other erythrocytes, of course, are injured by the immersion of the animal in the lead solution, but the formation of crystals is usually restricted rather narrowly to the periphery of the drop. The cytoplasm is apparently injured sufficiently to affect the union of the hemoglobin with it, but the permeability of the cell membrane is not enough modified to permit the escape of the hemoglobin, and consequently the slight increase in concentration, brought about by momentary drying, results in crystal formation.

The failure of these cells to accumulate neutral red, even when exposed to higher concentrations than normally used, is another evidence of modification of the membrane and perhaps of the cytoplasmic contents as well. A similar explanation of crystal formation could be offered in the laking experiments described by Stewart ('18), in which the hemolytic agents (saponin or sodium taurocholate) and the blood of Necturus are placed on the slide in small drops and then mixed.

Quite frequently in the preparation of blood smears, which are fixed by drying only, erythrocytes are encountered in which one or several refractive bodies of varying size may be observed. These bodies have been observed in both urodele and reptilian blood and are quite striking objects in an otherwise satisfactory smear. Similar observations have been reported for the red corpuscles of the rat by Adams and Shevket ('29). These observers found that such refractive bodies, usually single in the rat, do not react to neutral red, Janus green, brilliant cresyl blue, or to Wright's stain; nor do they correspond to the descriptions of the Howell bodies or refractive body of Isaacs ('25). While the refractive body of the rat corpuscle was found in all of the cells of one slide, none might be seen on a similar slide made at the same time from the blood of the same animal. It was considered to be an artifact or a degeneration phenomenon due to technique.

In view of preceding observations on intracellular crystallization of hemoglobin in Necturus erythrocytes, it is suggested that these refractive bodies appearing in certain smears of blood represent incipient centers of crystallization which have been sufficiently modified by technical procedures to conceal the true nature of their origin. They are apparently induced by unconscious variations in the technique, such as thickness of the blood film and consequent variations in the rate of drying.

Similar granules were described by Guerber ('27) in the erythroblasts of pig and calf embryos and were also interpreted as 'Kristallembryonen.'

SUMMARY

Intracellular crystallization of hemoglobin may be readily induced, in the erythrocytes of Necturus previously poisoned by prolonged immersion in lead acetate, by the momentary drying of drops of blood on the slide before covering. Only the peripheral cells in contact with the glass are so affected and they may accordingly be found distributed in a circular pattern in the covered preparation.

Apparently all or only part of the hemoglobin within a cell may become crystallized. The number of crystals formed in each cell varies, one, two, and three being the most common.

Even in cells not stained supravitally, the slender filamentous mitochondria may become visible as the cytoplasm clears during the formation of a crystal. In supravitally stained preparations the neutral-red bodies and mitochondria are more easily observed and in some cases a few of these elements appear to become incorporated within a crystal.

Crystal formation is associated with a greatly decreased viscosity of the cell contents, the cell membrane becomes conspicuous, and in many instances the crystals become large enough to cause great distortion of the cell.

High concentrations of neutral red do not induce reticulation in these modified cells.

It is suggested that certain of the refractive bodies found in smears of blood represent incipient stages of intracorpuscular crystallization of hemoglobin.

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PLATE 1

EXPLANATION OF FIGURES

All drawings were made from sealed preparations and were outlined by means of a camera lucida. The finer details were filled in free hand; mitochondria solid black, neutral-red bodies as circles, and hemoglobin crystals in heavy outline.

1 An erythrocyte supravitally stained with neutral red and Janus green, showing the characteristic distribution of mitochondria and neutral-red bodies within the cell. The hemoglobin is only partially crystallized. Note the deformation of the cell on the side next the crystal.

2 A similar cell stained only with neutral red.

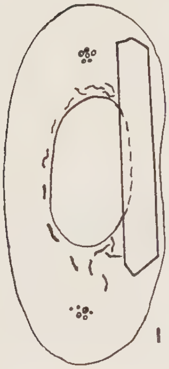
3 An erythrocyte prepared as in figure 1, also showing partial crystallization of the hemoglobin.

4 A similar cell, stained with neutral red, showing a crystal lying at right angles to the long axis of an erythrocyte. Crystallization of hemoglobin is incomplete.

5 An erythrocyte stained only with neutral red. Crystallization of hemoglobin was apparently complete and the unstained mitochondria were clearly observed.

6 Erythrocyte stained with neutral red and Janus green, containing a large crystal which deformed the cell and displaced the nucleus.

7 to 12 Several erythrocytes drawn twenty-four hours after the formation of the crystals, illustrating the variations in size, position, and number of crystals. Figures 8, 9, and 10 were drawn from preparations stained with neutral red, while figures 7, 11, and 12 were drawn from unstained preparations. In these cells all of the visible hemoglobin was in crystalline form.



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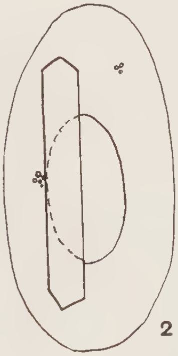

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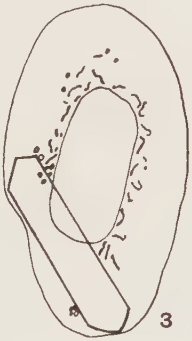

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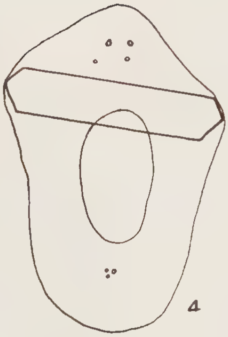

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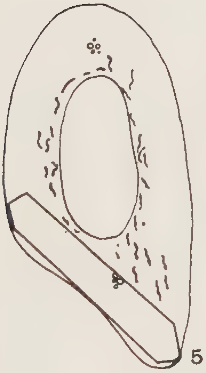

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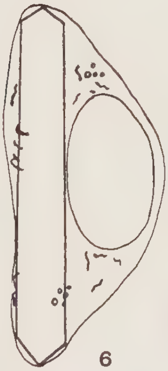

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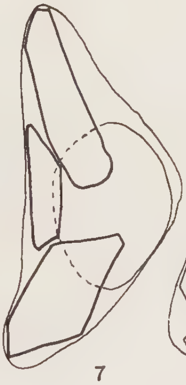

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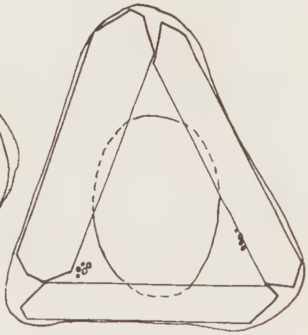

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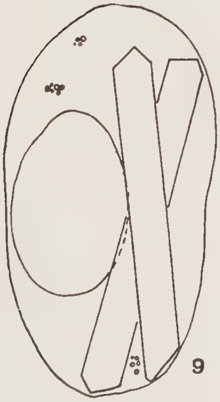

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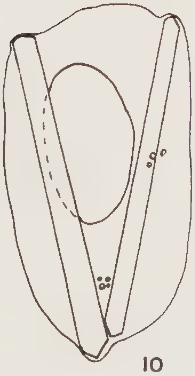

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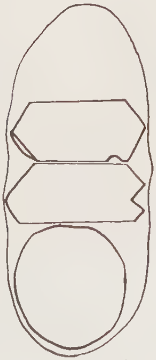

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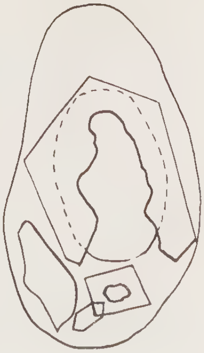

12

AN IMPROVED METHOD FOR THE COLLECTION OF RAT PLASMA FOR TISSUE CULTURE

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TWO FIGURES

The medium most commonly used in tissue culture in vitro is chicken plasma, but it is often necessary to use the mammalian plasma of the rat. The use of rat plasma involves two complications which have discouraged many from following this procedure: first, the difficulty of collecting the blood; secondly, the rapid clotting time.

The most common methods of collecting blood are the heart method and the carotid method. The heart method consists of drawing blood from the heart by means of a syringe. Besides requiring the more difficult technique of opening the chest, this operation in itself induces cyanosis, which results in decreasing the clotting time of the blood collected. Time is lost in the transference of the blood from the syringe to the centrifuge tube. A more or less violent agitation of the blood during this process also has a tendency to reduce clotting time through the release of thromboplastic substances. The method of Lambert and Hanes in which the carotid is cut and the spurting blood collected directly into the centrifuge tube does not prevent the admixture of tissue juices which greatly accelerates the clotting time.

We have experimented for some time with the methods mentioned. Contrary to the statement of Lambert and Hanes that cannulization in the rat is out of the question, we have developed a technique which we find most efficient. The use of the cannula precludes the possibility of admixture of tissue juices and enables the operator to collect the blood directly into the centrifuge tube with the least possible amount of agitation and exposure. We have cannulized the abdominal aorta, since it is the largest and most accessible artery in the rat. In our experience this method has yielded approximately three times as much blood as either the heart or carotid method. Moreover, plasma thus obtained by our technique will last for approximately three hours with only ice used to retard clotting.

DESCRIPTION OF TECHNIQUE

A. Apparatus

A cannula and a special tube for storing the plasma and embryo juice are the only pieces of apparatus necessary besides the ordinary tissue-culture apparatus.

The cannula used is made by drawing out a glass tube of 7-mm. diameter so that its outside diameter is 1 mm. This tube is broken by hand into 8-cm. and 10-cm. lengths. By hand breaking, beveled edges are formed which facilitate the

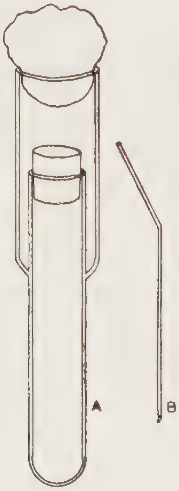

Fig. 1 A. Diagram of double-necked tube used for storage of plasma or embryo juice. B. Cannula used for obtaining blood.

insertion of the tube into the artery. This beveled edge is made smooth by fusing it in a flame. At about 3 cm. to 4 cm. from the other end of the tube an obtuse angle is made by gently heating the capillary tube and allowing the segment to bend about 30°.

We have also devised a special tube for storing the plasma and embryo juice. It consists of a small tube 7.5 cm. in length, 1.5 cm. in diameter, rounded off at one end. A collar is fused to it about 2 cm. from the open end and projects 3 cm. beyond the tube, giving a total length of about 5 cm. For ice storage the opening of both the inner and the outer tube may be stopped, thus doubly insuring sterility and at the same time allowing direct contact between the ice and the inner tube. Therefore it is unnecessary to store the tube of plasma within another tube as described by Strangeways ('26) and others. While in use the tube is supported at an angle of 45° in a beaker of ice and may be opened and closed many times without flaming and yet remain sterile. In this case only the cotton plug is used, the inner stopper being dispensed with.

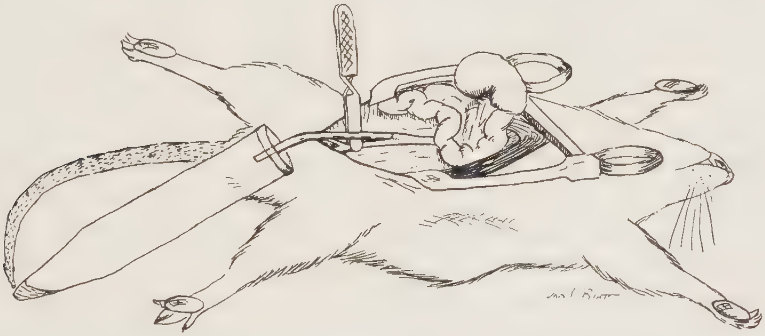

Fig. 2 Diagram of rat, showing abdomen opened and cannula inserted into aorta for the collection of blood. Note that cannula need not be tied in.

B. Method of obtaining blood

The abdomen is opened under strict aseptic conditions. The embryos are then removed to be used for the preparation of cultures and embryo juice. A small self-retaining abdominal retractor is inserted and the stomach and intestines are lifted out of the abdomen, thus exposing the inferior vena cava and the abdominal aorta. The posterior peritoneum is then cut with a fine scissors. With two pairs of small forceps the posterior peritoneal fat and fascia are separated from the aorta and inferior vena cava. Starting at the bifurcation of the aorta, an area of about 2 cm. is cleaned. The inferior vena cava is not separated from the aorta, as this process is apt to produce hemorrhage. Two small bulldog clamps are placed on the aorta about 1.5 cm. apart and the intervening portion of the aorta bathed in sterile paraffin oil. Midway between the two clamps a V-shaped incision is made with a fine pair of scissors, the apex directed toward the head. The small flap formed is now lifted with a fine forceps and the opening in the aorta is thus exposed. The beveled end of a cannula previously sterilized in paraffin oil is inserted into the opening of the aorta toward the upper bulldog. The bulldog is then removed and the cannula is pushed beyond this point. The blood will now spurt out of the cannula, and after discarding the first few drops, it is collected in an iced, paraffined, centrifuge tube. The blood is then centrifuged for five minutes and the supernatant plasma transferred with a pipette to the special double-necked tube. If the plasma tube is kept on ice and used with the precaution mentioned below, it will last for at least two to three hours.

C. Preparation of the cultures

A drop of embryo juice is placed on each of three cover-slips, then a small piece of tissue is embedded in each drop. An ice-cold pipette is used to draw up three drops of plasma. One drop is placed near each of the three drops of embryo juice. This pipette with any remaining plasma is discarded, since its reintroduction into the plasma will cause a clot to form much sooner. The two drops are now quickly mixed and a clot begins to form by the time the third culture is completed. The cover-slips are then mounted.

Because of the relatively large quantity of plasma obtained by the above method, we have found that as much as half of it usually remains unused. This can be saved for transplants by the addition of heparin, provided the growth is to be used for cytological purposes, and not for pharmacological experiments where its use has been found inadvisable. Erdman has also reported this same objection to the use of heparin. In some of the cytological work we have used E. C. Craceum's method of adding heparin directly to the blood before centrifuging and have dispensed with the use of ice and the vacuum bottle entirely. The method has proved very satisfactory.

DERMATOGLYPHICS IN TWINS OF KNOWN CHORIONIC HISTORY, WITH REFERENCE TO DIAGNOSIS OF THE TWIN VARIETIES

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INTRODUCTION

This note is concerned with an attempt to differentiate monochorionic and dichorionic twins solely on the basis of the dermatoglyphic features, the epidermal ridge configurations of the finger tips, palm, and sole. Though the material comprises only eight pairs of twins, it affords a significant test of the dermatoglyphs as diagnostic criteria, for the reason that the evidence of the fetal membranes is available as a check. It may be stated here that, as indicated by the membranes, the series contains four pairs of monochorionic twins and four pairs of the dichorionic variety.

The familiar twin problem needs no detailed restatement, excellent discussions and literature reviews being available in several recent publications (see especially Komai, '28; Newman, '28 a, '28 b; Reichle, '29 a, '29 b; Siemens, '27; Verschuer, '27). It is only necessary to recall the distinctions of the two varieties of twins. In the first class are included those pairs produced from a single fertilized ovum. This origin predicates the formation of a common chorion, as well as close resemblance of the two individuals. Such twins have been variously designated, with emphasis accorded to one or another of their distinctive features: monozygotic, uniovular, monovular, single-ovum—monochorionic—identical, duplicate, similar, homologous. In this paper the term monochorionic will be employed, the term being chosen in preference to monozygotic and its synonyms to give emphasis to the known chorionic relations of the twins under consideration; either designation is superior to expressions which state or imply that the twins are actually identical. Twins composing the second class are the product of concurrent development of two fertilized ova; they are usually credited with similarities and differences equal in kind and degree to those obtaining in brothers and sisters born at different times (with the apparent exception of certain curious reversals of asymmetry in crown-whorl and handedness—Lauterbach, '25; Verschuer, '27). Their fetal membranes are dichorionic, with possible occasional exceptions. The names applied to this variety stand in contrast to the terminology of monochorionic twins, emphasizing origin, chorionic relations, and resemblance: dizygotic, biovular, double-ovum—dichorionic—fraternal, dissimilar, heterologous. Herein they are called dichorionic twins, the term again emphasizing that the membranes are known and not committing itself with respect to degree of resemblance.

Attempts to distinguish monochorionic and dichorionic twins, apart from reference to their fetal membranes, have been based largely upon observation of physical traits. Twin pairs of unlike sex are at once recognized as dichorionic. In the like-sexed pairs, which may be either monochorionic or dichorionic, diagnosis is made in accordance with the degrees of resemblance and the existence of reversals of asymmetry. Discordant conclusions respecting the possibility of accurately rating twins have been reached by various writers, the opinions ranging from an optimism granting virtual infallibility in diagnosis to positions which deny the possibility of classifying twins. It is quite generally recognized, naturally, that assurance in diagnosis should increase in proportion to the comprehensiveness of the diagnostic scheme. Among the various schemes which have been employed, that of Siemens ('27) is to be noted especially. Dermatoglyphics figure prominently in a large share of the published twin studies, and by some workers are considered the most useful of the bodily traits for twin diagnosis (Komai; Newman). Lauterbach

('25), Reichle, and others, on the contrary, conclude that little dependence is to be placed upon these features, and it may be mentioned that Reichle's material includes several conjoined twins, in which monozygotic origin is indisputable, as well as normal twin pairs whose afterbirths he had examined. To anticipate, it may be said that the results now reported furnish additional evidence against the reliability of the dermatoglyphics as diagnostic aids.

MATERIAL

The material, comprising prints of eight pairs of twins, was placed at my disposal by Dr. A. H. Schultz, of the Laboratory of Physical Anthropology in the Johns Hopkins Medical School. The twins, born in the Obstetrical Department of that institution, were followed up in after years by Doctor Schultz for physical measurements and other observations. Ranging in age from two to fourteen years (table 2), the individuals are much more favorable for detailed study of the dermatoglyphs than are newborn infants, which might be assumed to serve most satisfactorily as subjects for correlated study of the dermatoglyphs and fetal membranes. Though it is true that both prints and fetal membranes may be secured from such cases, practice proves that young infants yield prints which are far from suitable for detailed examination. Granting that afterbirths may be obtained readily enough, difficulties attend the subsequent follow-up of the individuals, yet this procedure remains the only alternative if coordinated study of the fetal membranes and dermatoglyphs is to be realized. An appreciation of all these obstacles increases my sense of indebtedness to Doctor Schultz for his gift.

The prints are the usual form of ink impressions, including for each individual the finger tips, palms, and soles. The set of prints of each individual is recorded on a large sheet having spaces for the separate impressions (essentially duplicating the "Beobachtungsblatt" published by Schlaginhaufen, '12). The sheet bears Schultz's original notation of the name, race, sex, and age of the subject, as well as the symbol 'A' or 'B' distinguishing the members of a twin pair and the afterbirth observation. This system of records obviates confusion, both as regards the separate prints of one individual and the identities and records of the twins. The quality of the palm and sole prints is excellent on the whole, each print being complete and clearly decipherable. The prints of one pair of twins (III), however, are inferior, due to lack of cooperation on the part of the young subjects, to the fineness of the ridges, and apparently also to an excessive application of ink. The finger prints are imperfect technically in all but three pairs of twins, as a result of too heavy inking. While it has been possible to make dependable observations of the general features of the finger prints in six pairs, ridge counting proved to be so difficult that attempts were abandoned except in the three pairs mentioned.

Interpretation and formulation of the prints followed the methods of Galton ('92) for finger-tip patterns, of Cummins et al. ('29) for the palms, and of Wilder (in Wilder and Wentworth, '18) for the soles. It must be recognized that the formulae have their limitations in describing many of the characteristics which are considered in the twin comparisons, and that the comparisons must be made with the actual prints. The formulae, however, do give a partial picture of the configurations, and the list of formulae in table 1 may be relied upon to that extent as records of the resemblances and differences found in the twin pairs.

Passing from the twin subjects to their fetal membranes, it may be remarked that the entire structure of this study rests upon the assumption that twins derived from a single ovum are invested by one chorion, while biovular twins are dichorionic. The reservation must be made, however, of the possibility that a monochorionic relation may be established rarely in biovular twins. Reichle discusses fully the contention of Siemens, Verschuer, and others that the membranes are not dependable indicators of zygosity, and even dismisses the necessity of the reservation just mentioned.

TABLE 1

Dermatoglyphic formulae of the eight pairs of twins, stated in the customary symbols. The letters A and B identify the members of a pair; r, right; l, left; M, monochorionic; D, dichorionic

PAIR	IDENTIFICATION	PALMAR MAIN LINES	AXIAL TRIRADII	PALMAR PATTERNS	FINGER-TIP PATTERNS	PLANTAR PATTERNS
	Ar	10.9.6.5′.	t′	L^r/A^c.O.O.L.M.	U.U.U.U.U.	O.O.U.O.
I	Al	11(10).7.7(6).5′h.	t′	L^r/A^c.O.O.M.L/M.	U.A.A.U.U.	B.O.U.O.
M	Br	11.9.7.5′.	t′	A^u/L^u.O.O.L.M.	U.U.A.U.U.	O.W.O.O.
	Bl	11(10).7.7(6).5′h.	t′	L^r/A^c.O.O.M.L/M.	A.U.A.U.U.	O.O.U.O.
	Ar	9.0.5″.5′.	t′	A^u/A^c.O.V.O.M.	U.U.U.U.U.	A.O.O.U.
II	Al	9.X.5″.2.	t′	L^r/A^c.O.O.V.V.	U.R.U.U.U.	W.O.O.U.
D	Br	9.0.5″.5′.	t′	A^u/A^c.O.O.O.M.	W.U.U.U.U.	W.O.U.W.
	Bl	9.0.5″.4.	tt′	A^u/L^u.V.O.O.D.	W.W.U.U.U.	W.O.U.O.
	Ar	9.7.5″.5″.	tt′	A^u/L^u.O.O.O.L.		W.O.U.O.
III	Al	9(10).7.5″(6).5″.	t	A^u.O.O.O.L.		W.O.U.O.
M	Br	11.0.7.5″.	tt″	L^u.O.O.O.O.		W.O.U.O.
	Bl	11.9.7.5′.	t	A^u.O.O.L.O.		W.O.U.?
	Ar	10.9.6.5′.	t	L^r.O.O.L.D.		A.∩.U.O.
IV	Al	11(10).9.7(6).4.	t	L^r.O.O.L.D.		A.∩.U.O.
D	Br	9–7.9.5″.5′.	t′	A^u/A^c.O.D.L.D.		W.∩.W.O.
	Bl	9–7.0.5″.5′.	t	A^u.O.D.O.D.		W.W.W.O.
	Ar	7(8).5″(6).5″.4.	t′	A^u/A^c.O.O.O.L.	W.U.U.W.U.	A.O.U.U.
V	Al	7.5″.5′.3(2).	t′	A^u/A^c.O.O.O.L.	W.R.W.W.W.	A.O.U.U.
D	Br	7(8).5″(6).5″.3.	t′	A^u/A^c.O.O.O.L.	W.U.U.W.U.	W.O.U.U.
	Bl	7.5″.5′.2.	t′	A^u/A^c.O.O.O.L.	W.U.U.W.U.	W.O.U.O.
	Ar	11/7.0.7.5′.	t′	A^u/A^c..O.O.V.D.	W.W.W.W.W.	A.∩.W.U.
VI	Al	11/7.0.7.5′.	t′	A^u/A^c.O.O.V.D.	W.W.W.W.W.	A.∩.W.O.
D	Br	9/7.9.5″.5′.	t′	A^u/L^u.O.O.L.D.	U.U.U.U.U.	A.∩.O.O.
	Bl	9/7.9.5″.2.	t′	A^u/L^u.O.O.L.D.	U.U.U.T.U.	A.∩.O.O.
	Ar	11.9.7.4.	t	L^r.O.O.L.M.	U.U.U.U.U.	A.O.W.O.
VII	Al	9.7.5″.3.	tt′	A^u/L^u.O.O.O.L/M.	U.R.A.U.U.	A.O.W.O.
M	Br	10/7.9.7/6.3.	t′	A^u/A^c.O.M.L.D.	W.W.U.U.W.	A.O.U.O.
	Bl	9/7.X.5″.4.	t′	A^u/A^c.L.O.O.D.	W.W.U.W.W.	A.O.U.O.
	Ar	11.X.7.5′.	t′	A^u/A^c.O.O.V.M.	U.U.U.U.U.	A.∩.U.U.
VIII	Al	9–7.X.5″.3.	t′	l^r/A^c.O.O.O.D.	W.T.T.U.U.	A.∩.U.U.
M	Br	11/7.9.7.3.	tt′	L^r/A^c.O.O.L.D.	W.R.U.W.U.	A.∩.U.U.
	Bl	9–7.7.5″.3.	t′	A^u/A^c.O.O.O.D.	W.W.U.U.U.	A.∩.U.U.

Conclusions based upon the chorionic histories of twins naturally demand authentic observations of the fetal membranes. As pointed out by Reichle, routine hospital records are most often not dependable with regard to this point, either because attention is not directed to the only positive criterion of diagnosis—the composition of the septum separating the two amniotic cavities—or, if a diagnosis is stated, evidence of its validity is wanting. The afterbirths of the twins here considered were examined by Dr. J. Whitridge Williams, whose experience in the matter justifies confidence in the membrane diagnoses.

DIAGNOSES

The writer had requested of Doctor Schultz, when the material was proffered, that all data giving clues to the varieties of twins be withheld, pending diagnoses based upon the dermatoglyphs alone. In consequence of this request, the sheets of prints came to my hands with the data (written on the sheets themselves) covered by pasted papers. The data remained covered during the first studies of the prints, of which the results are presented at this point. It will be clear, therefore, that the diagnoses are unbiased by knowledge of the sex, chorionic relation, or other relevant data.

In diagnosing the twins the effort was made to apply Newman's procedure ('28 a, pp. 291, 292) with as much strictness as possible, though it is evident that influences from other sources were introduced (see discussion of pair V, below). The writer must confess that his decisions in certain of the cases were made with considerable doubt, but since the crucial point of the test is the assignment of each pair to one or the other variety, it was necessary to review repeatedly the items under comparison and to arrive at an unqualified diagnosis.

The diagnoses are listed in table 2. It will be noted that there is conflict with the membrane observations in two pairs, III and VII, which are called dichorionic, in disagreement with the observed membranes. More exacting analyses of the prints, described in following pages, show that the dermatoglyphic differences in the members of those two pairs would justify the stated diagnoses if the criteria for differentiating monochorionic and dichorionic twins are to be accepted as valid. Even if it were granted that pairs III and VII are actually dizygotic and that their monochorionic state is deceptive, other evidences of failure of the diagnostic criteria are to be demonstrated in the following pages. The experience in diagnosing pair V may be mentioned in this connection; these twins are dichorionic and thus certainly dizygotic. It was only by a chance clue that the pair was correctly diagnosed, and I may add that if the diagnostic criteria had not been violated in this case, the pair would have been recorded, incorrectly, as monochorionic. A remarkably close resemblance exists in their dermatoglyphs, as shown in later tables. Breaking the rules of diagnosis, I discounted the resemblances on the ground that the dermatoglyphs (the palms especially) conform to a type which is very common in the negro, the dermatoglyphs in this race being subject to a smaller range of variation in the particular features than is commonly met in racial collections. The incident illustrates that factors additional to degree of resemblance and bilateral comparisons may warrant attention in dermatoglyphic studies of twins no less than in consider-

TABLE 2

Data pertaining to the eight pairs of twins, together with diagnoses based upon the dermatoglyphs. M, monochorionic; D, dichorionic

PAIR	RACE	AGE, YEARS	IDENTIFICATION LETTER AND SEX	CHORION	DERMATOGLYPHIC DIAGNOSIS
I	White	14	A♀; B♀	M	M
II	Negro	4	A♀; B♂	D	D
III	White	2	A♂; B♂	M	D
IV	Negro	5	A♀; B♂	D	D
V	Negro	6	A♂; B♂	D	D
VI	Negro	4	A♀; B♀	D	D
VII	White	8	A♀; B♀	M	D
VIII	White	6	A♀; B♀	M	M

ing other bodily features. Being at the time unaware of the race to which pair V belongs (it is actually negro), the writer might have been misled as readily by the clue of racial variation. In the white peoples, where the traits observed in this twin pair are relatively much less frequent, their occurrence might have an opposite significance, perhaps increasing the chance of a monozygotic relationship.

The score of only six correct diagnoses in eight cases (table 2) indicates that differentiation of the twin varieties presents difficulties which should not be minimized. The two recorded errors in diagnoses consist in interpreting twins which are actually monochorionic as of the dichorionic variety, and in addition an instance is cited illustrating chance avoidance of error in another pair, actually dichorionic but with characteristics which would stamp them as monochorionic according to the diagnostic scheme.

RATING THE TWINS ACCORDING TO DEGREES OF RESEMBLANCE

It must be remembered that a large number of features are examined in the process of comparing a pair of twins, and that it is necessary both to avoid giving disproportionate weight to the more outstanding elements of the configurations and to construct some sort of record by which the numerous observed points may be summated. In his diagnoses the writer employed such a record, and the conclusions were based upon summation of the resemblances and differences therein listed. In view of the discrepancies revealed in the diagnoses, it was considered essential both to extend the comparisons and to place them on a more objective and rigid basis.

The dermatoglyphs lend themselves equally to qualitative and quantitative comparisons. Finger prints, for example, may be first classified according to the familar pattern types (whorls, loops, etc.); also there may be applied the ingenious methods of Bonnevie ('24) which determine the shape of such patterns (circular or elliptical) and their 'tendency to twisting.' With the addition of Bonnevie's 'quantitative value,' determined by ridge counting, a complete expression of qualitative and quantitative characteristics of the apical patterns is attained. The palm and sole together present a series of nine pattern areas which are readily accessible for study, and the configurations in these areas vary widely. In addition, the palm possesses four main lines, as well as such special features as axial triradii which may be included in the comparisons. The configurations of the palm and sole are not so well adapted to precise quantitative determinations as are the finger prints, though certain measurements of the palmar dermatoglyphs have been chosen as representing points of comparison which are similarly significant.

TABLE 3

Quantitative values of the finger prints in three pairs of twins. M, monochorionic; D, dichorionic

PAIR	CHORION	A		B	
		Right	Left	Right	Left
I	M	8	5.5	6	4
V	D	26.5	30	26	25.5
VII	M	14.5	13.5	29	32

Quantitative values of finger prints

The finger prints, as mentioned in the discussion of material, are in the main unfavorable for accurate ridge counting. Counts could be made in only three pairs of twins, for conversion into the quantitative value of Bonnevie. The result, contained in table 3, may at first seem discordant with Bonnevie's statistical findings in study of the quantitative value in twins. Working with thirty-one pairs of same-sexed twins, Bonnevie reports:

> Out of these twins, ten pairs (Group IV) should, without doubt, be considered as being only fraternal, because of their very slight facial similarity. Thirteen other pairs (Groups II and III) with a more conspicuous similarity have been grouped as being questionable, the resemblance being, however, in seven of them (Group II) so great that these should very probably be considered as identical twins. This is the case also with the remaining eight pairs of my

> twin material (Group I), in which the facial similarity is very striking. Thus, although no definite information has been obtained with regard to the mono- or dizygotic origin of the twins, I shall probably not be wrong in considering the 15 last-named pairs (Groups I and II) as representing identical twins, while the other sixteen pairs (Groups III and IV) are looked upon as fraternal twins. . . . [Bonnevie's table] proves a close correspondence to exist between the general resemblance (identity?) of the two members of a twin pair and the resemblance also of their quantitative pattern-values. A comparison of the twin pairs of Group I with those of Group IV gives a clear demonstration of this fact, while the more questionable groups, II and III, with regard to the quantitative values, illustrate transitions between these two extremes.

Notwithstanding this demonstration, together with the high degree of correlation found in identical twins by Bonnevie, the fact remains that the classifying of individual pairs of twins in accordance with their resemblance in quantitative values presents serious difficulties. The likeness in values displayed in pair I would suggest that this pair is monochorionic, which it is known to be, yet the members of the dichorionic pair V likewise have very nearly equivalent values, and the known monochorionic twins of pair VII have quite dissimilar values. Comparable individual discrepancies occur in Bonnevie's material. That the quantitative values of the finger-tip patterns are associated with total resemblances and differences in other dermatoglyphic features is suggested by the quantitative and qualitative comparisons of the palmar and plantar configurations in the same twins as shown in following tables.

Measurements of the palmar dermatoglyphs

In an unpublished study of bimanual variation (Cummins, Leche, and McClure) measurements of the palmar dermatoglyphs have been employed for the first time. Four of the measurements are intertriradial intervals, the distances between triradii a–b, b–c, c–d, and a–d. A fifth determination indicates the elevation of triradii b and c from a line joining a and d. The sixth measurement gives the 'line-D index,' an expression of the radial extent of transversely coursing ridges in the distal region of the palm, line D having been recognized as an indicator of transversality of the distal ridge system. Two measurements are taken to record the position of the most proximal axial triradius, expressing its shifting proximally and ulnarward.

The foregoing measurements were made for each set of twins, and compared in the six possible couplet combinations of the four hands: A right with B right, A left with B left, A right with B left, etc. In the comparison the absolute values were first reduced to quotients, the measurement of A being assigned the numerator place in every instance:

$$\frac{A}{B} \times 100$$

The substitution of quotients for the actual values renders it possible to evaluate the differences in terms of deviations from equality (100). Thus the differences revealed in corresponding hands of the twins may be compared directly with the differences in opposite hands of the twins or with bimanual differences in the same individual. For each such comparison I have averaged the deviations from equality in the nine measurements; inasmuch as twin A invariably forms the numerator (so arranged that in the analysis it was always clear which twin had the larger measurement), the quotients deviate from 100 in both directions.

A certain weakness in this procedure will be apparent, though indeed the same weakness appears in twin comparisons of any sort, whatever the character of the features compared. Reference is made to the fact that each of the several measurements receives equal weight in calculating the average differences. It seems probable that the features may be of differential value, though there is no means at hand of determining these values. The situation has a parallel in comparisons of other somatic characteristics.

Employing the average differences, the twins may be rated according to degree of resemblance. Since the factors of parallel imaging and mirror imaging must be taken into account (Newman), it is clear that the rating should be based upon both homolateral and heterolateral comparisons; bilateral comparisons in the individual twins are to be reserved for later consideration. Table 4 shows negligible difference in the results of the homolateral and heterolateral comparisons. For rating the twins according to their total resemblance, therefore, it is not unwarranted to employ the averages of the differences shown in the homolateral and heterolateral comparisons, as presented in table 5. The result indicates that diagnoses based upon resemblance in the dimensional features would be subject to error. While it happens that the two end positions, representing closest and least resemblance, are occupied respectively by monochorionic and dichorionic pairs, one dichorionic pair (V) in the upper range of the list is insignificantly different from the monochorionic pair leading the table; it is followed, moreover, by another dichorionic pair, while three sets of monochorionic twins are found low in the rank of resemblance.

TABLE 4

Homolateral and heterolateral comparisons of the twins in each pair, together with bilateral comparisons in the individuals. Numerical values explained in text

	PAIR	QUANTITATIVE DIFFERENCE			QUALITATIVE DIFFERENCE		
		Homolateral	Heterolateral	Bilateral	Homolateral	Heterolateral	Bilateral
Mono-chorionic	I	4.4	2.6	3.4	21.3	28.1	31.9
	III	13.4	14.6	11.8	38.6	41.4	27.1
	VII	12.9	13.2	12.6	46.9	48.8	24.4
	VIII	8.9	11.1	11.8	26.9	28.1	26.3
Dichorionic	II	8.1	7.3	8.5	35.0	46.3	24.4
	IV	14.0	14.0	5.5	51.8	52.7	20.9
	V	3.7	4.7	3.8	20.6	27.5	21.3
	VI	19.6	20.6	6.9	61.9	61.9	10.0

Non-metric comparisons of the dermatoglyphs

Sixteen features have been chosen for the comparisons next described: course of line A; reduction of line C; the five palmar pattern areas—hypothenar, thenar/first interdigital, second, third, and fourth interdigitals; the five apical patterns of the digits; the four distal plantar areas—hallucal and three interdigitals. Line B is not included for the reason that its variations are so intimately associated with the character of the neighboring interdigital configurations, which are separately catalogued, and the consideration of line C is limited to the state of reduction for the same reason. Other features of the palmar dermatoglyphs, including line D, are adapted to measurement, and are thus dealt with in the preceding section.

TABLE 5

Ranking of the eight pairs in the order of increasing difference in quantitative and qualitative comparisons. Figures explained in text. M, monochorionic; D, dichorionic

PAIR	MEMBRANES	QUANTITATIVE DIFFERENCE	QUALITATIVE DIFFERENCE	MEMBRANES	PAIR
I	M	3.5	24.1	D	V
V	D	4.2	24.8	M	I
II	D	7.7	27.5	M	VIII
VIII	M	10.0	40.0	M	III
VII	M	13.0	40.6	D	II
III	M	14.0	47.9	M	VII
IV	D	14.0	52.4	D	IV
VI	D	20.1	61.9	D	VI

The object in these non-metric comparisons is to obtain a rating of the differences existing in the twin pairs, for the present excluding the individual bilateral comparisons as in the instance of the metric comparisons. In the features now to be considered it is difficult to apply the strictly objective treatment to which dimensional features are fitted. For example, in comparing the hypothenar configurations the two hands may be found alike, either in the total absence of a pattern or in the existence of the same pattern in both; on the contrary, only one hand may possess a patterned configuration while the other bears an open field, or both may have patterns, but of unlike configuration. Each of the features listed is subject to such variations. It is necessary to arrange a schedule for the record of the differences. Fully appreciating that the sixteen features may not be entitled to equal weight in a summated statement of differences, yet finding no means of determining a correction for such inequalities, like values are assigned to all. This difficulty is pointed out in connection with the treatment of measurements. After trials of various schemes, it was decided to express the comparison of each feature in terms of an arbitrary numerical value, 5 representing total unlikeness and with 0 standing for identity. Intermediate grades of difference were to be represented by the figures 4, 3, 2, and 1. For each feature the scale of values was determined by the nature of the variations to which that feature is subject (as they had become familiar in previous work with some 1200 individuals). It is to be emphasized that these values are employed only as working records, and are based solely upon anatomical comparisons. Bonnevie has pointed out in her work with finger prints that various characteristics of these patterns may be of unequal weight genetically. Having arranged the schedule of values, comparisons are made in each pair of the four couplet combinations of hands (homolateral and heterolateral combinations). Totaling the result for the sixteen features, each having a maximum value of 5, complete unlikeness in each couplet comparison would be indicated by the value 80. It is considered desirable to substitute for this figure a base value of 100, hence the total differences observed in each comparison are converted accordingly; the figures in round numbers range from 10 to 62, and they are to be interpreted on the basis of 100 as the sign of total unlikeness. In pair III, where the finger prints and sole prints are indecipherable, and in pair IV, lacking the finger-print determinations, the values of the available features are weighted to give totals comparable to those of the sets in which the prints are complete.

In tables 4 and 5 the total differences in the non-metric comparisons are presented in company with the average differences in measurements. It will be noted that the scale of differences compares closely with the differences shown in the non-metric comparisons. Again the conclusion is presented that the twins cannot be reliably classified on the basis of resemblance.

BILATERAL COMPARISONS IN THE INDIVIDUALS

In accordance with the suggestions of Komai and Newman, it is apparent that the inquiry should next include a weighing of the bilateral difference in the individuals against the results of homolateral and heterolateral comparisons in the two twins. For this purpose the bilateral differences observed in the twins of each pair are averaged in the same manner as the couplet combinations of hands for homolateral and heterolateral comparisons. The figures are presented in table 4.

In this series of twins there is no consistent difference in the results of the two comparisons. In the instance of metric comparisons, with the exception of two sets of dichorionic twins (IV and VI), the bilateral differences and total difference of the twins are of equivalent grade. In the two pairs named the bilateral differences are markedly exceeded by the total differences of the twins. These two sets of twins, it should be noted, lie at the extreme of dissimilarity (table 5). The non-metric comparisons show even less consistency in this respect, inasmuch as the monochorionic group is not characterized by close agreement of bilateral and intertwin differences. Dichorionic pairs II, IV, and VI repeat the relationship shown only by the last two pairs under metric comparison, while two monochorionic pairs (III and VII) are added to the group showing bimanual differences of less magnitude than the differences between the two twins of each pair.

It should be especially interesting to examine separately the variability of line D, a feature in which characteristic bimanual differences have been long recognized. In right hands, in mass analysis, this line extends farther radially than in lefts, indicating a greater development in the radial direction of a transverse ridge system in the distal area of the palm. While the distinction naturally is often illustrated in single pairs of hands, frequent exceptions make it necessary to treat line D in the twins as individual cases in which its behavior may have, after all, no association with the state of twinning. The line-D indices are listed in table 6, and these call for a word of explanation. The larger the index, the more pronounced is the radial extension of the transverse ridge system. In other words, increase in the size of the index is to be taken as an expression of the tendency which is demonstrated statistically on the part of line D to extend farther radially in right hands. In comparing the indices a difference of 5 points or less probably lacks significance; a difference of 10 points or more is of unquestioned significance. Thus one may compare the right and left hands of each twin, to determine whether there is a significant inequality, and the two twins may be compared for indications of suggestive differences.

TABLE 6

The line-D index in the eight pairs

	PAIR	A		B	
		Right	Left	Right	Left
Monochorionic	I	77	78	72	77
	III	59	60	80	86
	VII	85	55	60	57
	VIII	75	55	75	59
Dichorionic	II	53	58	59	44
	IV	79	79	63	62
	V	36	30	31	30
	VI	88	90	60	62

Allowing for insignificant numerical differences in the indices, the twin pairs may be assorted into four groups in accordance with their illustration of the statistical trend of line D. 1) Parallel imaging, in which the right and left hands of both twins display the typical bimanual distinction of line D: pair VIII, monochorionic. 2) One twin having on both hands a line D which is characteristic of the statistical trend of either right or left hands, while the other twin shows the characteristic bimanual difference: pair II, dichorionic; pair VII, monochorionic. 3) Bimanual distinctions wanting in both twins: pair I, monochorionic; pair V, dichorionic. 4) One twin showing on both hands the trend either of right hands or lefts, while the other exhibits on both hands the statistical trait of the opposite: pair III, monochorionic; pairs IV and VI, dichorionic. This comparison fails to separate the monochorionic and dichorionic twins of the series.

TABLE 7

Comparison of the total differences of the eight pairs with the average bilateral differences of the twins in each pair. Twins arranged in the order of increasing total differences. Figures explained in text. M, monochorionic; D, dichorionic

PAIR	MEMBRANES	TOTAL DIFFERENCE	AVERAGE BILATERAL DIFFERENCE	RATIO OF BILATERAL DIFFERENCE
I	M	19	21	111
V	D	20	16	80
VIII	M	32	30	94
II	D	35	25	71
III	M	46	31	67
VII	M	48	30	63
IV	D	52	18	35
VI	D	68	15	22

For summarizing, the bilateral differences the twin pairs may be ranked in the order of increasing difference. Table 5 shows, as before mentioned, that there is a fairly close agreement in the seriations of the twins arranged separately for the measured and qualitative features. Since there are some inconsistencies in the two orders, it is convenient now to construct a ranking based upon weighted averages of the figures representing quantitative and qualitative differences. These averages are presented in table 7, accompanied by comparable averages of the bilateral differences in each pair of twins.

Two analyses of the figures are suggested. First, the absolute bilateral differences should be examined with reference to their distribution among the twin varieties. In general, the magnitude of bilateral difference is higher in the monochorionic group, though pairs I and II must be pointed out as exceptions. It is perhaps true that such differences may have a meaning in mass treatment of a large body of twin material, but that they could be relied upon in diagnosis of individual twin pairs seems questionable. The second analysis of the figures suggests that the magnitude of the bilateral differences may be a function of the collective dermatoglyphic variation of the individuals, an expression of variation which is necessarily confined by the character of the particular configurations, and which may be entirely independent of the fact that the individuals are twins. Included in table 7 there is a column presenting the relative magnitude of the bilateral and intertwin differences, expressed as indices. Notwithstanding that disorder is produced in the array of absolute values of the bilateral differences when they are placed in the sequence of increasing total differences of the twins, the relative magnitudes arrange themselves in a decreasing progression, broken only by pair V. The seriation demonstrates that the twin varieties are not to be differentiated by the relative magnitudes of bilateral and intertwin differences.

It will be of interest, further, to inquire into the bilateral differences in the individuals of each pair, to show their separate contributions to the average differences listed in table 4. From approximate equality in the amount of bilateral difference, comparing the twins of each pair, the twins range in the following order of progressive limitation of the differences to one twin of the pair: I$^{(M)}$, II$^{(D)}$, VII$^{(M)}$, VIII$^{(M)}$, VI$^{(D)}$, V$^{(D)}$, III$^{(M)}$, IV$^{(D)}$. In pairs I and II the two twins show bilateral differences which are practically equal in value; pairs III and IV represent extreme reduction of bilateral differences in one twin of each pair, while the remaining four pairs are intergrades uniting the extremes in the order listed. While monochorionic and dichorionic twins are mingled in the seriation the tendency of clustering should be noted. The clustering is correlated neither with the absolute bilateral differences (averaging the two twins) nor with the total differences between the twins. Though it may have a significance in statistical analysis of a large series, the feature hardly fulfills the requirement of a diagnostic criterion.

SUMMARY AND CONCLUSIONS

The dermatoglyphs (comprising finger tips, palms, and soles) are analyzed in eight pairs of twins whose fetal membranes had been examined by an experienced observer. The aim in the study is to determine whether resemblances and differences in the dermatoglyphic features provide an adequate means of differentiating monochorionic and dichorionic twins, the observations of the afterbirths serving as a check. Conceding the possibility that a single chorion may occasionally occur in twins which are actually dizygotic, the writer believes that the conclusions here stated are still tenable.

Diagnoses of the twins are made without knowledge of the membranes, sex, or other data which might influence judgment in the dermatoglyphic examination. In two of the eight pairs of twins the dermatoglyphic diagnoses disagree with the chorionic findings, the two pairs being erroneously diagnosed as dichorionic. In another pair strict adherence to the diagnostic scheme would have introduced an incorrect diagnosis of twins which are actually dichorionic. In view of these discrepancies, an attempt is made to render the dermatoglyphic comparisons more objective and rigid, as a measure for further checking of these features against the known chorionic types. Toward this end, measurements of eight features on the palm, together with anatomical characteristics of sixteen configurations of the finger tips, palm, and sole, are reduced to numerical expressions by which the twins may be ranked according to the magnitude of their total differences. This ranking, which is based upon homolateral and heterolateral comparisons of the twins in each pair, is supplemented by a similar determination of bilateral differences in the individuals, for the comparisons suggested by

Komai and by Newman. Neither the ranking of the twin pairs according to degree of total differences nor the ranking of bilateral differences leads to an assortment by which the monochorionic and dichorionic twins are separated. The conclusion is therefore forcibly presented that the dermatoglyphs, as here analyzed at least and in this series, fail to supply a secure basis for the diagnosis of individual pairs of twins. It may be added that A. H. Schultz, the donor of the sets of prints, has published certain body measurements of twins which lead him to the same conclusion respecting size differences as diagnostic criteria; in a personal communication Doctor Schultz informs me that the series on which he has reported ('26) includes the twin pairs whose prints are here studied.

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HUMAN FINGER TYPES

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TWO FIGURES

SUBJECTS OF STUDY

In the present investigation measurements were made of 620 hands, of which 402 were males and 218 females. All of the subjects were white adults, mostly medical students in the University of Toronto, supplemented by other students, members of the staff, militia recruits, and friends of the writer. No one was measured who had a history of fracture or dislocation of either the index, ring, or middle fingers.

In the human hand the middle finger is the longest, the thumb is the shortest, and the little finger is the next shortest. The relative lengths of the index and ring fingers make a disputable point, and to elucidate it the present investigation was undertaken.

Authorities differ as to the relative lengths of the index and ring fingers. Wood Jones ('20) and Schultz ('24) state that the index finger is usually longer than the ring finger and Wood Jones describes the long index finger as a 'characteristic human specialisation.' Baker ('88), on the contrary, found the ring finger to be the longer, though he adds that ''the index finger sometimes equals the ring finger and rarely exceeds it.'' Ecker ('74), working with negroes, found the ring finger to be longer in males and the index finger to be longer in females. Mantegazza ('77) found ''over 500 with a longer ring finger, men predominating, and under 100 with a longer index finger, women predominating.'' A similar observation was made by Pfitzner, as quoted by Schwalbe ('90).

METHOD OF MEASUREMENT

A finger board, as illustrated, was devised to measure the relative lengths of the index and ring fingers, using the tip of the middle finger as a datum point. This board is made from a piece of hardwood about 30 × 11 × 2 cm. The slides and center stop are made as follows: Take a strip of well-dried hardwood, with a fine grain, measuring 56 × 2 × 0.7 cm. First bevel 0.2 cm. off one side and then cut off two pieces each 10 cm. in length. These are the flanking strips. The remainder will make the center stop and the two slides and it should therefore be beveled on the side opposite to the one already beveled. After this has been done, cut off a length of 10 cm. as the center stop and divide the remainder into two equal lengths as the slides. To each slide glue a scale as shown in figure 1. This scale may be made by cutting two lengths of 5 cm. each from a celluloid measure. These specifications for the dimensions of the center stop and the slides should be followed exactly, because experience has shown that these dimensions will fit the width of most fingers and avoid contact with an overhanging finger nail.

Before the adoption of this method of measuring, extensive trials were made of other methods. Hand outlines were traced both by the subjects themselves and by an individual investigator, but it was found that the outlines were not accurate. Hence the adoption of the finger board, which is simple to make, quick and easy to use, and gives accurate results.

The finger board is used as follows: The subject places the palm of the hand flat on the board with the skin of the tip of the middle finger touching lightly the near end of the center stop. The forearm should be flat on the table on which the board rests and the forearm directly in line with the middle finger. This position is shown in figure 1 and should be copied exactly. If this position is not copied exactly, the measurements may be worthless, because a slight abduction or adduction of the hand causes a variation in the prolongation of the fingers. After the operator has made sure that

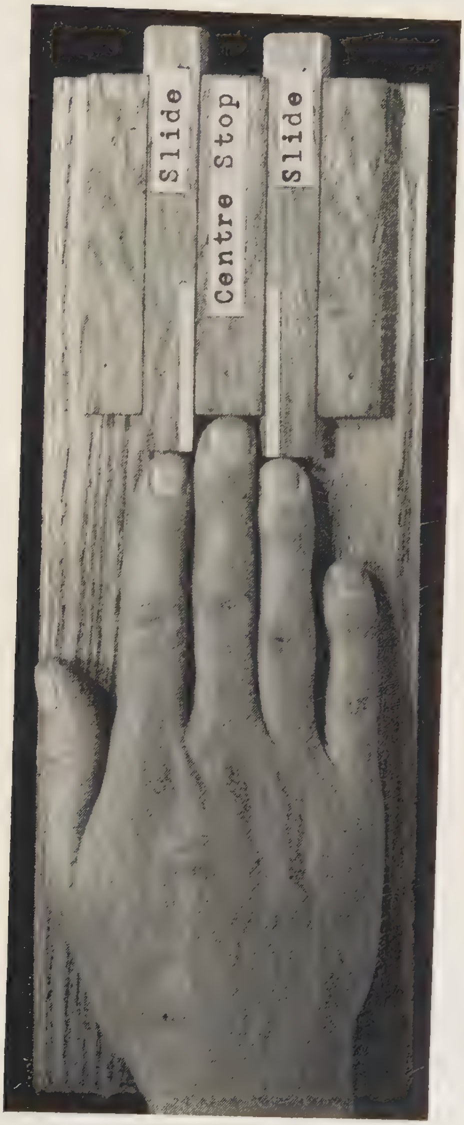
Slide
Centre Stop
Slide

Fig. 1 The finger board in use. The tip of the middle finger is placed against the end of the center stop and the graduated slides are adjusted to touch the tips of the index and ring fingers.

the hand is flat, the forearm in line, and the middle finger just touching the center stop, he adjusts the slides to touch lightly the skin of the tips of the index and ring fingers and notes the readings on the scales. The zero point of the scale is level with the near end of the center stop and the longer finger will consequently give the smaller reading on the scale. The readings are then recorded on a mimeographed form, of which figure 2 shows a completed sample.

The record includes a note of whether the subject was right-handed, left-handed, or ambidextrous. Eye color was also recorded as a rough indication of racial origin.

Name—*Jones, J.*
Sex—*Male*
Class—*Meds '34*
Eye color—*Blue*
Right-handed—*X*
Left-handed—
Ambidextrous—
Right hand:
  Index—*13*
  Ring—*10*
Left hand:
  Index—*11*
  Ring—*11*

Fig. 2 Record form for finger lengths.

The record forms are then sorted and the results tabulated as in table 1. If the readings given by the index and ring fingers are within 0.5 mm. of each other, the two fingers are taken as of equal length and so classified, because the technique is accurate only to within 0.5 mm.

RESULTS

Table 1 shows that in the right hands of males the majority (55 per cent) have a ring finger longer than the index. Only 28 per cent have a longer index finger and in 17 per cent both are of equal length. In the left hands of males the largest group (65 per cent) has a longer ring finger, only 20 per cent a longer index finger, and in 15 per cent both are of equal length. Taking both hands of males, the ring finger is longer in 60 per cent, the index finger longer in 24 per cent, and both are equal in 16 per cent.

In the right hands of females, the index finger is longer in 52 per cent, the ring finger longer in 24 per cent, and both are equal in 24 per cent. In the left hands of females, the index finger is longer in 47 per cent, the ring finger longer in 28 per cent, and both are equal in 25 per cent. Taking both hands of females, the index finger is longer in 50 per cent, the ring finger longer in 26 per cent, and both are equal in 24 per cent.

TABLE 1

Finger types

SEX	FINGER TYPE	RIGHT HAND		LEFT HAND		BOTH HANDS	
		Number	Per cent	Number	Per cent	Number	Per cent
Males	Index longer	56	28	40	20	96	24
	Ring longer	111	55	130	65	241	60
	Both equal	34	17	31	15	65	16
	Total	201	100	201	100	402	100
Females	Index longer	57	52	51	47	108	50
	Ring longer	26	24	31	28	57	26
	Both equal	26	24	27	25	53	24
	Total	109	100	109	100	218	100
Males and females	Index longer	113	37	91	29	204	33
	Ring longer	137	44	161	52	298	48
	Both equal	60	19	58	19	118	19
	Total	310	100	310	100	620	100

Taking both sexes together, the right hands show 44 per cent with a longer ring finger, 37 per cent with a longer index finger, and 19 per cent with both of equal length. The left hands show 52 per cent with a longer ring finger, 29 per cent with a longer index finger, and 19 per cent with both of equal length. Taking both hands of both sexes, the ring finger is longer in 48 per cent, the index finger is longer in 33 per cent, and both are of equal length in 19 per cent.

For the sake of brevity, the tabulations in reference to handedness and to eye color are not given, but there is no indication of any relationship between the finger type and either handedness or eye color.

CONCLUSION

In white adults the ring finger in males is generally longer than the index finger and in females the reverse is found. This is so both for the right hand and for the left hand. This confirms the observations of Mantegazza, Pfitzner, Baker, and Ecker and is contrary to the findings of Wood Jones and Schultz.

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THE RELEASE OF FOLLICULAR COLLOID FROM THE THYROID OF NECTURUS MACULOSUS FOLLOWING HETEROPLASTIC ANTERIOR-PITUITARY IMPLANTS

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THREE PLATES (THIRTEEN FIGURES)

The failure of Necturus to undergo metamorphosis becomes increasingly difficult to explain. As information concerning the morphology and physiology of the thyroid and pituitary glands of this species has increased, evidence indicating a normal endocrine equipment has accumulated. It therefore seems possible that the explanation of permanent neoteny in Necturus may eventually be found due to factors independent of its endocrine system.

Swingle ('22) showed that the Necturus thyroid is physiologically active when transplanted into the body of the tadpole. Charipper ('29) reported absolute increase in the size of the thyroid with increasing size of the animal, and in studies of the mitochondria and the Golgi apparatus he observed cytological evidences of restricted regional activity in the larval and adult glands. The same author ('30) presented experimental evidence showing that Necturus possesses a potent pituitary gland which when implanted into frog tadpoles causes precocious metamorphosis. In spite of these facts, Necturus fails to undergo metamorphic changes when it in turn is treated with thyroxin or anterior-lobe injections.

Information concerning the potency of the Necturus thyroid-pituitary apparatus is therefore based entirely upon a knowledge of the reactions which these glands cause when

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transplanted to the larval stage of some amphibian which normally undergoes metamorphosis, and there has been a complete lack of knowledge concerning the reaction of the Necturus thyroid within its own body.

The aim of the present study was to test the effect of anterior-lobe implants upon the Necturus thyroid. The investigation is one of a series of experiments undertaken for the purpose of analyzing the effect of anterior-lobe implantation upon the thyroid of different species of urodeles. The completed series will include similar experiments upon the thyroid of premetamorphic and postmetamorphic stages of different species of Amblystoma. The morphological picture of the thyroid thus obtained may be mapped against the histological pattern of the normal gland during the early diagnostic stages of metamorphosis previously recognized (Grant, '30).

This work was carried on under the direction of Prof. Alden B. Dawson, to whom acknowledgment is made for his interest and valuable criticisms, and also to Prof. William J. Crozier for his suggestions and encouragement.

MATERIAL AND METHODS

The experiments were carried on during March and April of 1930. Young Necturus larvae approximately ten months old and averaging 45 mm. in length were used. The animals, maintained under laboratory conditions after hatching, were raised on a diet of aquatic annelids and Enchytraeus.

The pituitary for implantation was obtained from male and female Rana pipiens. The animal was decapitated and the gland removed and placed in cold-blooded Ringer's solution. In this medium the anterior lobe was carefully separated from the other portions of the gland and the connective-tissue capsule covering it was removed.

The Necturus host was anaesthetized in a 1:3000 chloretone solution. The anterior lobe of the frog was cut into three or four pieces and inserted into the peritoneal cavity. The incision was closed by one suture made of a single strand separated from grade-0 white silk. Every step in the procedure was carried on under the binocular microscope with the aid of iridectomy scissors. Twenty-four hours after the operation, the animal showed normal feeding reactions. Because of the nature of the operation and the rapidity with which the reaction took place, it seemed evident that the response of the thyroid was induced merely by absorption of the anterior-lobe products through the body fluids of the host, and was not due to successful grafting of the implanted tissue.

The tissues were fixed in Helly or Champy fluid at different intervals following implantation, and serial sections, cut 5 μ in thickness, were treated with Mallory's triple connective-tissue stain. Material fixed with Champy was first treated with a haematoxylin stain (Heidenhain or Ehrlich) and followed by Mallory's technique.

This report is based upon the microscopic study of seventeen timed experimental cases and five control animals. The control specimens were killed at varying intervals throughout the course of the work, so that any morphological change due to seasonal or growth factors could be checked. Four of the control cases were animals on which no operation was performed, and the fifth received two implants of frog muscle tissue, forty-eight hours intervening, and was killed five days after the first implant.

HISTOLOGY OF THE NORMAL GLAND

The thyroid gland of Necturus has been described by Charipper ('29) as 'fundamentally bilateral.' Each main mass of colloid vesicles adheres closely to the mediocaudal surface of the first ceratobranchial cartilage. Cephalad to this mass of the gland small strands of isolated follicles are found close to other cartilaginous units of the hyobranchial apparatus.

The histology of the glands of the five control cases showed strikingly uniform pictures. The follicles as seen in cross-section varied in size from those of twelve cells to those of three (figs. 1 and 2). The lining cells were decidedly flat, with a homogeneous, uniformly staining cytoplasm. The colloid within the lumen of the follicles, in practically all of the vesicles, was deeply acid-staining and highly refractile and only rarely showed any non-stainable colloid. These properties of the colloid have been described by Uhlenhuth ('27), Hewer ('27), and Cramer and Ludford ('26) as characteristic of old, stored secretion.

In the serial sections of these ten normal glands only an occasional cell could be found containing any non-staining vacuoles and only one cell was seen to contain a colloid droplet. This corresponds to the histological picture described by Charipper and interpreted by him as evidence of the most restricted regional activity.

The histology of the normal thyroid of the larval Necturus may be described as that of the typical inactive storage gland. Uhlenhuth ('27) described the same histology in the larval glands of several urodeles which normally metamorphose and termed it the retention stage ('Stadium des schlauchartigen Follikel'). In other words, it is a gland in which the balance between the storage and release of colloid is held at such a low potential level that any morphological expression of the activity of the gland appears in only a very few cells which are located only after careful searching through serial sections of several glands.

HISTOLOGY OF THE THYROID GLAND FOLLOWING ANTERIOR-LOBE IMPLANTATION

Twelve hours after one implantation

Within at least twelve hours following the implantation of one anterior pituitary, striking histological changes were seen in the majority of the follicular cells, which, however, remained cuboidal and often flat, except in those whose shape was greatly distorted by large non-staining vacuoles (figs. 3 and 4). In addition to a striking vacuolization of the cells, a few intracellular colloidal droplets were also present. The follicles, still distended with stored colloid, retained therefore their general form, and the stored colloid still stained strongly acid and exhibited the highly refractile appearance of old secretion. The majority of the follicles showed a pattern similar to that of figure 3. Figure 4 represents a follicle in which the reaction was somewhat more extensive than in others of the twelve-hour cases.

Twenty-four hours after one implantation

The changes which have been described as appearing after twelve hours' implantation were present in a much more marked degree at the end of twenty-four hours. The cells were slightly columnar; the vacuoles were still present, but were of smaller size; and the intracellular content of colloid had markedly increased (figs. 5 and 6). In fact, at least one or two cells in the majority of the follicles (fig. 6) appeared so packed with discrete, colloid droplets that the cell cytoplasm could scarcely be seen. The follicular colloid retained for the most part the physicochemical properties of the old material. That the amount of secretion within the lumen was clearly reduced may be seen by comparing the quantity of stored material in figures 2 and 6. The follicle shown in figure 6 was larger and retained less colloid within its lumen.

Forty-eight hours after one implantation

A study of figures 7 and 9 will show the microscopic picture of the forty-eight-hour stage. The cells had become tall columnar in shape and within the cytoplasm were small vacuoles and numerous, fine, irregular particles of colloid having the appearance of an emulsion of colloid in cytoplasm. The secretion within the lumen showed still further reduction in amount and striking changes in its physicochemical properties, for it had lost its highly refractile appearance and had changed to a substance less solid in composition and was now basic in its staining reaction. The majority of the follicles corresponded to figures 7 and 9. An occasional one was seen similar to the portion of the follicle shown in figure 8, which resembled those of the twenty-four-hour stage, and contained one cell still filled with large, discrete, highly refractile colloid droplets, while the neighboring cell contained ‘emulsified’ colloid within its cytoplasm. A regional decrease in the density of the stored colloid is also indicated.

Seventy-two hours following one implantation

Many follicles of those glands which had been fixed seventy-two hours subsequent to anterior-pituitary implantation showed complete collapse of the vesicle and an absence of any follicular lumen and stored colloid (fig. 10). A fine ‘emulsion’ of colloid within the cytoplasm of the cells or a few, small, intracellular colloidal masses were the only evidences of colloidal material in such follicles. Figure 11 was drawn from a large follicle in which the lumen had not completely disappeared and the small core of the stored colloid still retained the hyalin appearance of old secretion. Many large, discrete, intracellular droplets of colloid were still present, but the ‘emulsification’ of the colloid was limited to the apical end of the cells.

Frequent implants over long periods

Figures 12 and 13 show follicles from a gland removed from an animal three days following the first implantation and twenty-four hours after the second. The pattern may best be described as that which might be obtained by superimposing the picture shown in figure 4 or 6 upon figure 9, which was, in fact, the actual condition of the experiment. The thyroid had received a second stimulus while in a state of response to the first implant, and the gland accordingly showed a morphological picture which was obviously one of increased activity. The large non-staining vacuoles in the colloid stored within the lumen have previously been described by numerous authors as characteristic of the hyperactive thyroid. The distribution of chromophobe vacuoles in figure

13 closely resembles many illustrations given by Uhlenhuth ('27) for the thyroid of the metamorphosing urodele. Figure 12 shows a completely collapsed follicle drawn from the same animal. Other animals which had received two, three, or four implants over three-, six-, or eleven-day periods showed follicles resembling figures 12 and 13.

DISCUSSION

From the foregoing description it is clear that the Necturus thyroid responded to anterior-lobe implants within at least twelve hours after the operation. The evidence of this response was seen in a change in its morphological pattern. A study of this timed series showed a progressive emptying of the stored colloid from the thyroid follicles into the blood capillaries which surround them. Complete collapse of the follicles appeared at the end of the third day. In other words, the only direct effect which anterior-lobe implantation brought about within the time of the experiments reported was the release of the stored colloid.

As a result of these experiments a theory of transcellular colloid release is presented for the following reasons:

1. Colloid was progressively released from the follicles of the gland.
2. The persistent morphological picture during this process was characterized by a rich, intracellular colloid content, first seen as large, discrete, highly refractile droplets and later as a fine 'emulsification' of the stored material which had gradually decreased in density.
3. Therefore, since the gland showed progressive emptying, it does not seem possible to interpret the colloid content of the cells as a product of synthesis, but on the contrary, it must be regarded as morphological proof of the transcellular method of colloid release.

The actual mechanism by which the cell takes up the stored secretion is difficult to discover and presents in itself many physical problems. Whether it resembles the absorption of fat by intestinal epithelium, or whether it is accomplished by some process resembling phagocytosis, is at the present unknown. However, it remains clear that whatever may be the mode by which the colloid enters and leaves the cytoplasm, the thyroid cells of Necturus, when stimulated by anterior-lobe implantations, transport the follicular colloid through their cytoplasm. The process begins within twelve hours by an absorption of the colloid material, which is seen as discrete droplets in the cytoplasm of the cells lining the follicle and appears in the same physical state as does the old stored colloid. As the process continues there is an obvious liquefaction of the stored secretion, which shows marked changes in its staining properties, so that as the colloid becomes less dense it passes through the cells in the form of an 'emulsion.' In other words, as the process of release of colloid continues, and as the properties of the stored colloid change in response to some activity, digestive or otherwise, of the epithelial cells, the intracellular colloidal content is seen to resemble the physicochemical state of the stored material.

This description of the process is contrary to previous theories advanced for release of colloid in the amphibian thyroid. Uhlenhuth ('27) reported intercellular release of colloid from the amphibian thyroid follicle during the period of normal metamorphosis of several urodele species. Hirschlerowa ('27) described a system of microscopic intercellular canals in the thyroid of the Anura. These spaces, which increased in number at the time of metamorphosis, she believed to be the special mechanism by which the gland poured forth its secretion into the blood capillaries.

Both of these authors failed to observe the amphibian thyroid during incipient stages of metamorphosis, criteria for which have recently been described by the author (Grant, '30). Until experiments similar to those described in this paper have been completed upon the thyroid of the premetamorphic and postmetamorphic stages of different species of Amblystoma, and until patterns of the thyroid obtained from these experiments are compared with the histological pictures of the thyroids of incipient metamorphic stages, no conclusive statement can be made concerning the method of colloid release in amphibians.

Florentin and Weis ('30) reported upon the secretory phenomenon in the thyroid of birds. In this group of vertebrates they described stainable secretory antecedents within the cytoplasm of the epithelial cells of the thyroid. These granules, larger at the basal part of the cell than at the distal pole, were passed into the lumen of the vesicle. A very unusual method for the release of colloid was described by these authors, who maintained that large groups of vesicles underwent complete disintegration and the follicular colloid was thus poured into the lymphatic system. Amitotic figures in the glandular epithelium and masses of degenerating cellular elements forming emboli within the lymph capillaries were cited by them as evidences of ''massive degeneration of large groups of glandular epithelium.''

Cells in the process of proliferation were not unusual in the experimentally stimulated Necturus thyroid (fig. 13), but they were not abundant, so that no significant interpretation was given to their presence in face of other more striking changes already described.

Reports upon the thyroid of mammals form the greatest part of the extensive literature dealing with thyroid studies. Bensley ('16) showed that the sensitivity of the mammalian thyroid to dietary conditions, temperature variations, seasonal changes, including hibernation, was undoubtedly the explanation of the many conflicting statements in the literature. The absence of a knowledge of the physiological condition of the thyroid prior to observation led authors to diametrically opposed conclusions regarding what were undoubtedly comparable processes.

Aron ('30) reported a method of colloid release in the guinea-pig, rabbit, and the dog which he analyzed from carefully controlled experimental studies of the thyroid following anterior-lobe injections. His theory of thyroid excretion in mammals compares so closely with the present description for Necturus that an extended citation of his studies seems necessary. Aron found the mammalian thyroid responded to anterior-lobe substances during the first day following injection of the material. From a study of follicles which were obviously in the process of colloid release he could observe no evidence of the secretion passing between the cells and was thus led to conclude that the stored colloid passed through the cytoplasm of the lining cells. As criteria of the release of stored secretion Aron recognized the partial loss of stainability of the follicular colloid, in the periphery of which appeared large numbers of chromophobe vacuoles. It seemed to him clear that the secretion was passing through the epithelial cells in an unstainable state and his interpretation of the chromophobe vacuoles he made clear when he termed them 'vacuoles de resorption.'

It seems evident, therefore, that in the mammalian gland the follicular cells may absorb the stored secretion and transport it in an unstainable form through the cytoplasm; while the thyroid of Necturus gives morphological proof of this transcellular method of colloid export.

SUMMARY

1. Histological studies were made of the Necturus thyroid following implantations of the anterior lobe of the anuran pituitary into the peritoneal cavity of ten months' old Necturus larvae averaging 45 mm. in length.

2. All control cases showed thyroid glands with a histological picture characteristic of the inactive gland, in which the cells were very low, cuboidal in shape, and possessed a homogeneously staining cytoplasm. The follicular colloid stained strongly acid and appeared highly refractile—characteristics of old secretion.

3. All experimental animals showed histological patterns of a thyroid very sensitive to stimulation from anterior-lobe products. The first morphological picture recognized as definite evidence of response to anterior-lobe implantation appeared within twelve hours and was characterized by the extreme vacuolization of the cytoplasm of the thyroid cells

and the presence of intracellular droplets of colloid. From the study of a series of cases killed and fixed at twenty-four-, forty-eight-, and seventy-two-hour intervals, it was evident that the follicles progressively released the stored colloid. At the end of the third day, the majority of the follicles were completely collapsed and empty. The persistent morphological picture through these timed series was an abundant intracellular colloid content, which first appeared in the form of large, discrete, highly refractile, colloid droplets, and later, as the stored secretion became reduced in amount and changed in its physical nature, the intracellular material appeared in the form of an emulsion of colloid in cytoplasm.

4. Since the follicles showed progressive release of stored colloid, and the correlated morphological pictures showed abundant intracellular colloid content, the thesis of transportation of stored colloid through the follicular cells of the Necturus thyroid is presented.

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EXPLANATION OF PLATES

Figures 1 to 13 are camera-lucida drawings of follicles in cross-section from the thyroids of Necturus larvae averaging 45 mm. in length. All figures were drawn with the same magnification (approx. × 1400) and details were added from oil-immersion study. The experimental conditions of the animal are indicated in each case and the follicles drawn were predominant and typical for that stage.

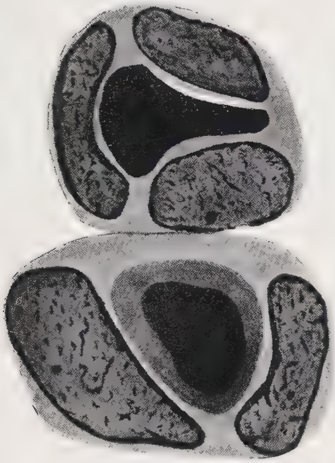
PLATE 1

EXPLANATION OF FIGURES

1 (Normal, control animal.) Two small thyroid follicles in cross-section. Flat epithelial cells showing a homogeneously staining cytoplasm. The follicular colloid appears deep acid-staining, dense, and highly refractile. The follicular colloid of the lower vesicle shows an outer rim which appears less dense in composition. Champy; Heidenhain's haematoxylin.

2 (Normal, control animal.) One large thyroid follicle in cross-section. Follicular colloid appears homogeneously dense in composition. Fat granules in the basal region of two cells. Champy; Heidenhain's haematoxylin and Mallory's connective-tissue stain.

3 and 4 (Twelve hours after implantation of one anterior pituitary.) Two thyroid follicles of different sizes in cross-section. Marked vacuolization of lining cells and a few intracellular colloid droplets. Champy; Ehrlich's haematoxylin and Mallory's connective-tissue stain.



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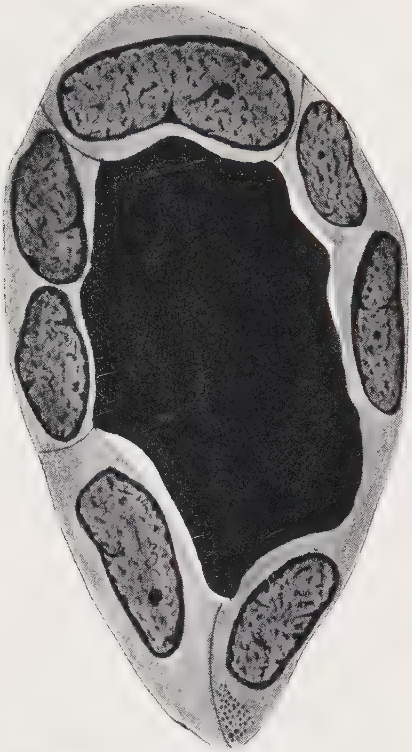

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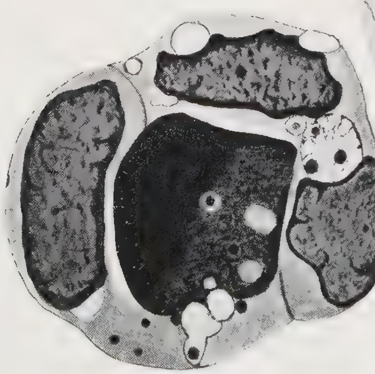

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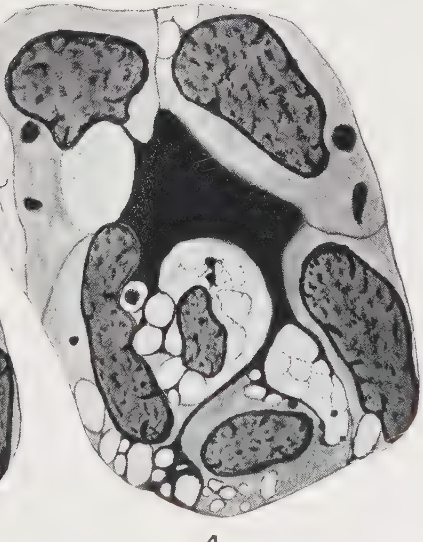

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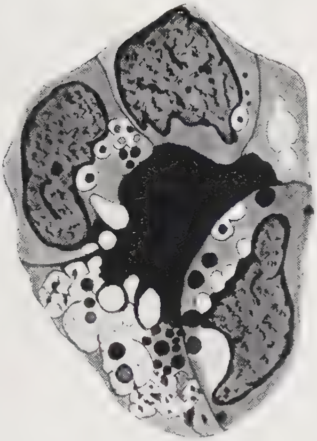
PLATE 2

EXPLANATION OF FIGURES

5 and 6 (Twenty-four hours after implantation of one anterior pituitary.) Two thyroid follicles of different sizes in cross-section. In comparison with the follicles of the 'twelve-hour stage,' the vesicles show a definite increase in the intracellular colloid droplets and a relative decrease in the amount of follicular colloid, which still appears highly refractile and dense. In figure 6 there are seven cells showing fat granules. Champy; Ehrlich's haematoxylin and Mallory's connective-tissue stain.

7 to 9 (Forty-eight hours after implantation of one anterior pituitary.) Two thyroid follicles of different sizes and two cells of a third follicle in cross-section. In figures 7 and 9 the follicular colloid appears less dense in composition, and in figure 8 a regional change in the physical state of the stored secretion is seen. Many of the epithelial cells are filled with numerous, very fine, irregular colloid particles suggesting the appearance of an emulsion of colloid in cytoplasm. One of the cells in figure 8 still shows numerous, large discrete droplets of colloid within its cytoplasm, while the neighboring cell contains 'emulsified' colloid. Champy; Ehrlich's haematoxylin and Mallory's connective-tissue stain.



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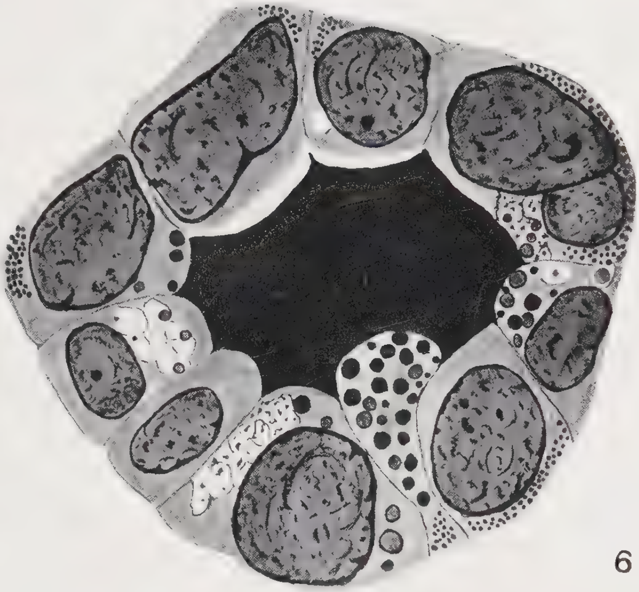

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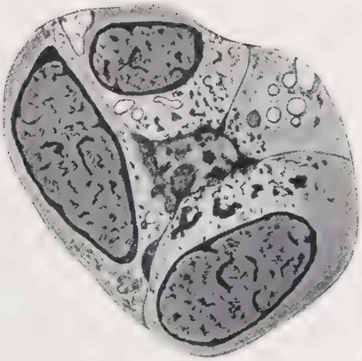

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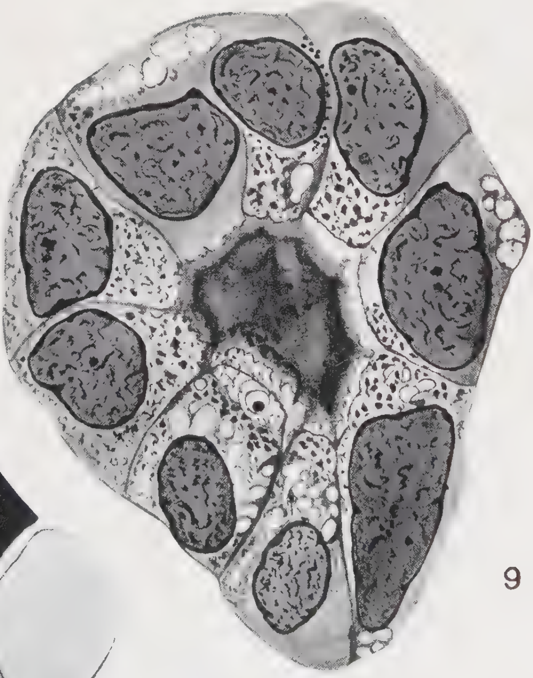

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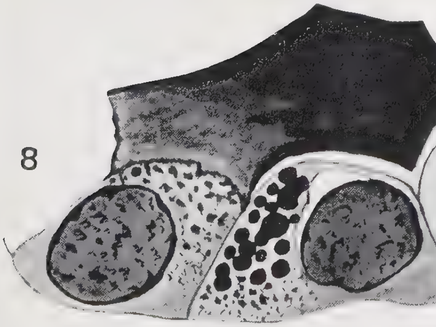

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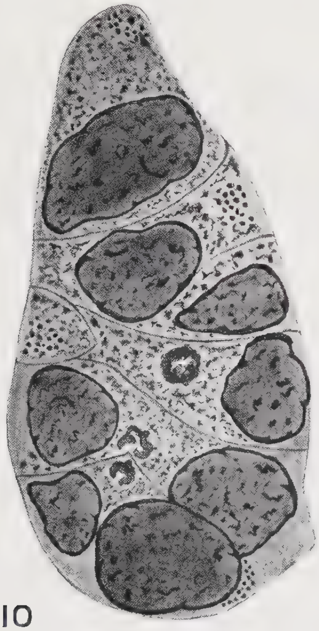
PLATE 3

EXPLANATION OF FIGURES

10 and 11 (Seventy-two hours after implantation of one anterior pituitary.) Two large thyroid follicles in cross-section. Figure 10 shows one follicle which had completely collapsed, with consequent absence of lumen and stored colloid. A fine 'emulsion' of colloid within the cytoplasm and three intracellular colloid masses are the only evidence of colloid material in this section of the vesicle. Four cells show fat granules at their bases. Figure 11 shows a section of a follicle in which the lumen had not completely disappeared and the small core of stored colloid still retains the hyalin appearance of old secretion. Many large, discrete, intracellular droplets of colloid are still present. The 'emulsification' of colloid is limited to the apical end of the cells. Champy; Ehrlich's haematoxylin and Mallory's connective-tissue stain.

12 and 13 (Twenty-four hours after successive implantations, forty-eight hours apart.) Figure 12, a completely collapsed follicle, with several cells containing unusually large masses of colloid. Figure 13, a vesicle in which the follicular colloid shows numerous chromophobe vacuoles, and appearing within the cells are numerous, discrete colloid droplets as well as 'emulsified' colloid. The numerous chromophobe vacuoles in the follicular colloid, and the large masses of intracellular colloid, well marked in figure 12, are taken to indicate a more rapid release of colloid following two implants than that observed from one implantation. One cell in figure 13 is seen in the process of mitosis. Helly; Mallory's connective-tissue stain.



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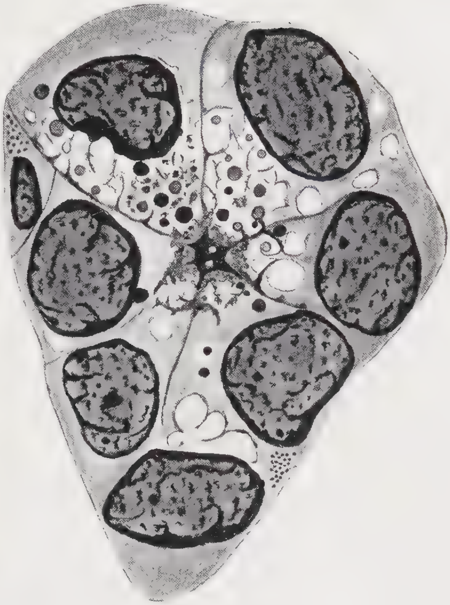

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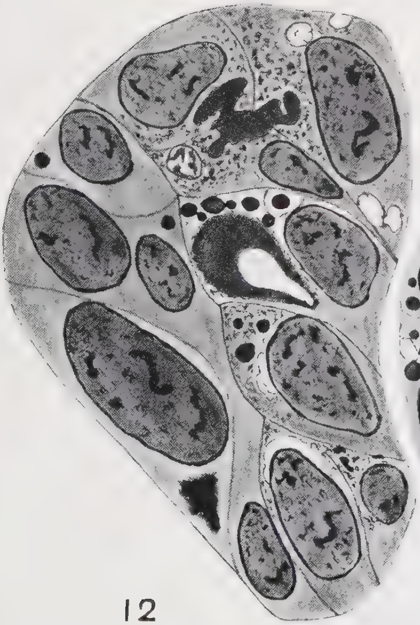

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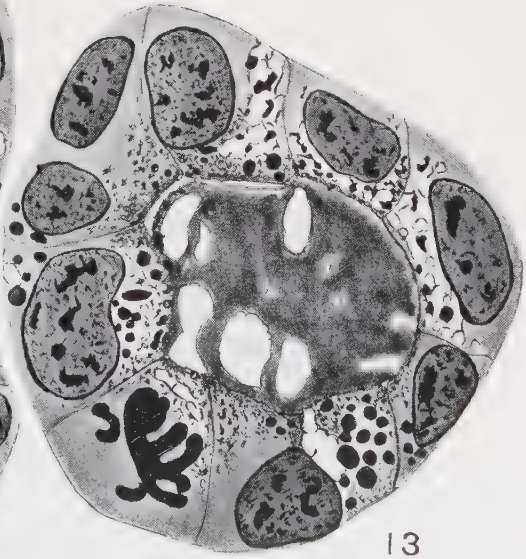

13

ORBITAL VARIATIONS

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SIX FIGURES

Muscle variations are so common in the anatomical laboratory and so many have been reported in the literature that one is reluctant to add to this number. In the orbit, however, relatively few have been described, hence they are still of anatomical interest. If one may judge from the number of inquiries received by those of us teaching graduate courses to specialists in ophthalmology relative to muscle variations, they are of more than passing interest to the clinician.

While it is undoubtedly true, as stated by Whitnall ('21), that gross anomalies of the orbital muscles are not as rare as would appear from the number recorded in the literature, yet even in his unusually large series of careful studies, if we exclude minor slips and slight variations in origin, he has been able to report but few major anomalies.

On the other hand, as we review the literature and note that the early anatomists, such as Vesalius, Albinus, and others, working under far greater difficulties than are found in any modern laboratory, were able to describe certain of these muscle anomalies, it is difficult to attribute their scarcity to poor laboratory conditions. Nor can one agree with Posey ('24) as to the lack of interest of anatomists in this region.

Certainly the statement of Lucien Howe, quoted from Posey ('24), does not apply to one of the anomalies to be described in this paper: "For it must be understood that none of these supernumerary muscles approach in size the other extrinsic muscles, but consist only of bundles of striped muscular fibers interwoven with connective tissue fibers."

Such excellent reviews of the literature can be found in LeDouble ('97), Whitnall ('11, '21), Hardy ('14), and Posey ('24) that a general review is unnecessary here.

For many years we required our students to make careful dissections of the orbits, asking that all variations be reported. During this period the writer also made many studies. In this large series but two major anomalies, a double inferior oblique and an unusual insertion of the superior oblique, were found. Recently, in a series of ten cadavers in which both orbits were dissected by the writer, he found in one specimen such an unusual number of anomalies as to merit a report. This specimen was a well-preserved adult female and anomalies were found in both orbits. The orbital contents were in as perfect a state as one would find in the fresh specimen.

LEVATOR PALPEBRAE SUPERIORIS

As would be expected from its position, some of the earliest muscle anomalies of the orbit to be recorded were of the levator palpebrae superioris. Vesalius, Albinus, and others described what is now known as the tensor trochleae of Budge ('59). This has possibly been most frequently reported. Bochdalek ('68) reported a transverse band, the musculus transversus orbitis. Whitnall ('21) reported three variations from the normal in one specimen, a tensor trochleae, a transverse orbital, and a retractor of the lacrimal gland. Rush and Schaeffer ('24) have described an accessory bundle to the reflected tendon of the superior oblique. In most of these the slips were small. A number of cases of absence of the muscle have been reported. Harmon ('09) described a clinical picture in a girl of ten years, which he assumed was due to a failure of the levator to separate from the superior rectus.

LEFT ORBIT

In the specimen referred to above, when the periorbita was removed from the left eye, the muscle mass of the levator palpebrae superioris, observed in its fascia, was far more extensive than normal. When the fascia was removed, what appeared to be a combination of the tensor trochleae of Budge and the band described by Rush and Schaeffer was observed (fig. 1, *A.M.*). This consisted of a muscle bundle 4 mm. wide emerging from the under surface of the levator palpebrae superioris about 1 cm. in front of the apex of the orbit. This bundle, as it passed forward and medially, was divided into two heads by a very large ophthalmic vein. The smaller lateral head, about 2 mm. wide, passed under the aponeurosis of the levator palpebrae superioris (fig. 1, *S.H.*). The large medial head, expanding to about 5 mm. wide, ended in a broad aponeurosis, apparently inserting into the trochlea and into the reflected tendon of the superior oblique (fig. 1, *L.H.*). The lateral horn of the levator appeared more massive than normal.

Reflecting the levator palpebrae superioris forward, this anomalous muscle bundle was found to be a part of a supernumerary muscle lying between the levator and the superior rectus (fig. 2, *A.M.*). This new plane of muscle was separated from the levator by a distinct layer of fascia, and was of greater volume than the normal levator. It arose from the apex of the orbit between the levator palpebrae superioris and the superior rectus, and consisted of three parts: 1) A large central belly passing forward above and almost completely covering the superior rectus (fig. 2, *R.F.*). At about the equator of the globe this expanded into an aponeurosis very similar to that of the levator, but entirely distinct except for delicate trabeculae. This aponeurosis inserted into the tunica propria at the fornix of the conjunctiva. Its lateral and medial horns passed to their respective bony walls, paralleling the horns of the levator palpebrae superioris (fig. 3, *A.Am.*) Traction on this central mass retracted the fornix of the conjunctival sac. The lateral and medial horns acted as distinct check ligaments against overretraction. 2) A small lateral slip of muscle (fig. 2, *R.L.*) passed almost horizontally outward from the tendon of the central mass to insert into the fascia of the lacrimal gland. 3) From the medial side arose the large head observed when the periorbita was removed. This, as described above, was divided into two parts by the superior ophthalmic vein (fig. 2, *S.H.*, *L.H.*), then joined to form a broad aponeurosis. This aponeurosis split around the trochlea and the reflected tendon of the superior oblique. Its medial fibers inserted into the bony wall above and below the trochlea. The remaining fibers formed a strong sheath for the reflected tendon of the

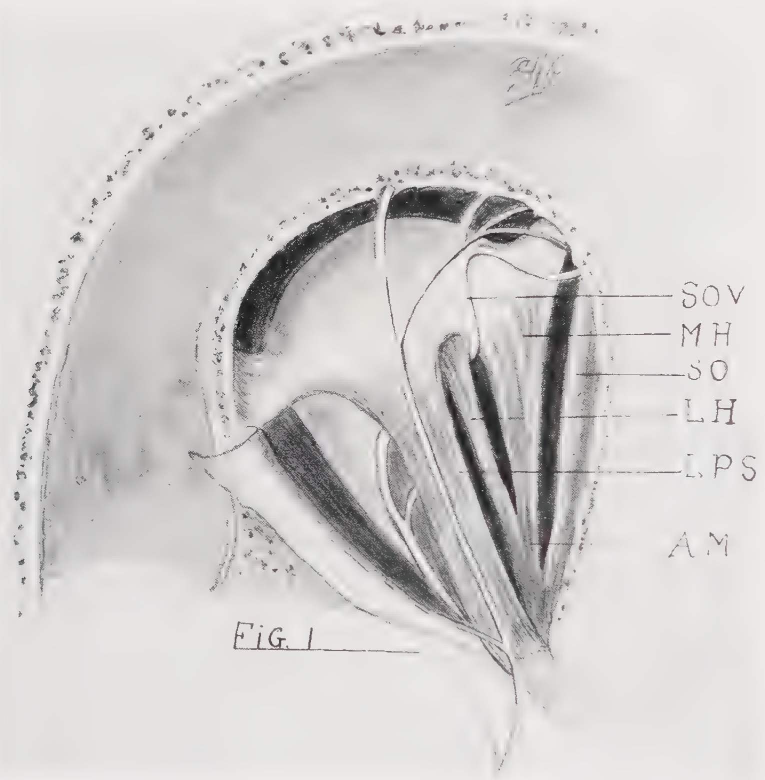

Fig. 1 Left orbit dissected from above. *A.M.*, accessory muscle; *S.H.* and *L.H.*, two medial heads of same; *L.P.S.*, levator palpebrae superioris; *S.O.*, superior oblique; *S.O.V.*, superior ophthalmic vein (reduced in all sketches and no branches shown).

superior oblique (fig. 2, *S.*). A fascial sheath separated this accessory muscle from the superior rectus. All the mass was supplied by the superior branch of the third nerve.

No other muscle variations were found in this orbit. The ophthalmic artery, however, passed medially beneath the optic nerve. This is not an uncommon variation—according to Quain's Anatomy (1894), in 15 per cent of the cases. It led, however, to three other vascular anomalies (fig. 3). First, the posterior external ciliary artery was the first branch from the main stem. Secondly, a small trunk arose medial and

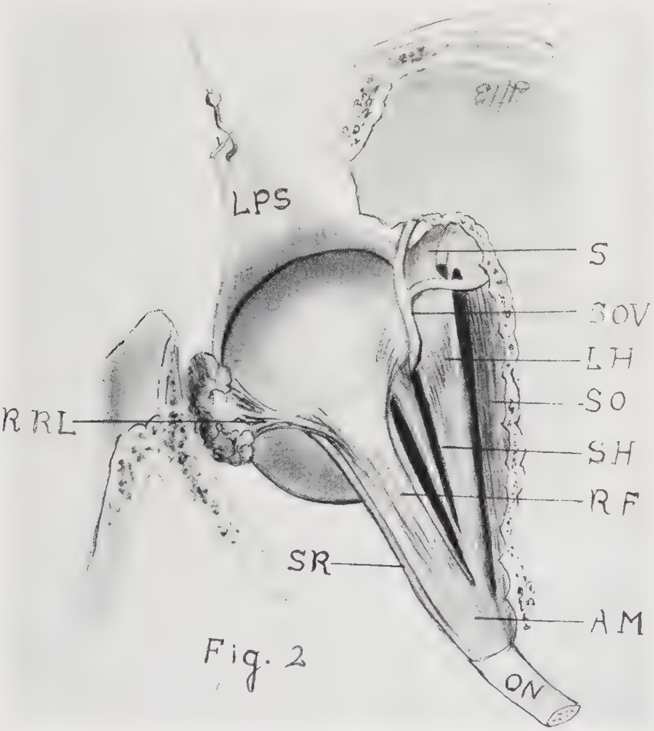

Fig. 2 Same orbit with the levator palpebrae superioris reflected to show entire accessory muscle (*A.M.*) and its four heads, *S.H.* and *L.H.*, medial heads; *R.F.*, retractor of fornix; *R.L.*, retractor of lacrimal gland. *S.R.*, superior rectus; *S.*, sheath.

superior to the posterior ciliary; this trunk gave off the arteria centralis retinae and then continued downward and forward to the inferior oblique muscle. Finally, the lacrimal artery arose from the main stem medial to the optic nerve and crossed over the nerve, beneath the superior rectus, then forward and laterally above the lateral rectus to reach the gland.

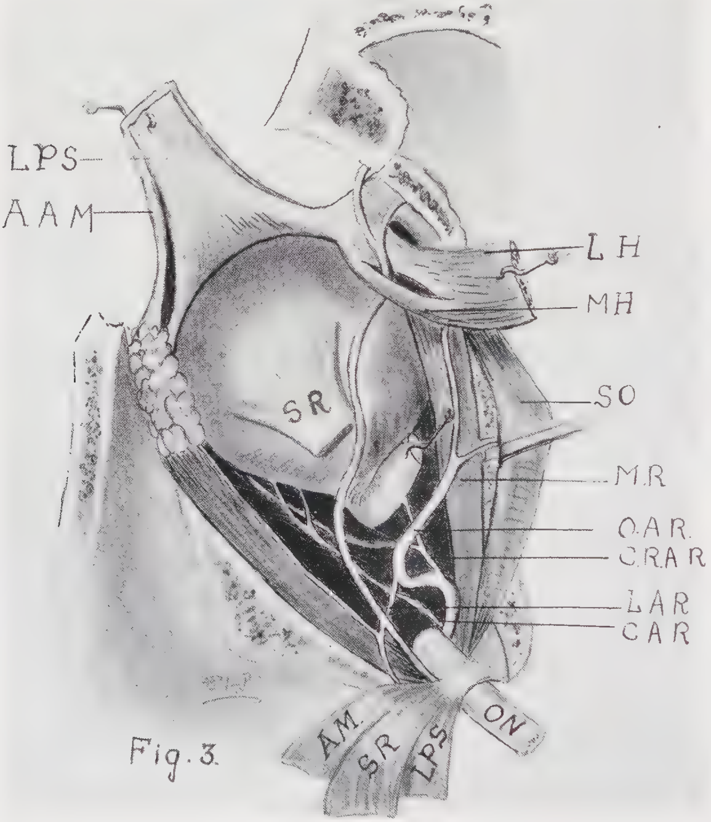

Fig. 3 Same orbit. Accessory muscle and its heads reflected to show its aponeurosis, *A.Am.; M.R.*, medial rectus; optic nerve reflected to show *O.Ar.*, ophthalmic artery; *C.Ar.*, external ciliary artery; *C.R.Ar.*, arteria centralis retinae; *L.Ar.*, lacrimal artery.

RIGHT ORBIT

The right orbit presented two muscle anomalies, a transverse orbital band and a variation in the insertion of the superior oblique. The transverse band (fig. 4, *Tr.O.*) differed from the one described by Whitnall in two particulars: first, that the convexity of the arch was in the opposite direction and, secondly, it was attached to the levator palpebrae superioris only on the under surface of the aponeurosis. On the lateral side of the aponeurosis a muscle belly 3 mm. wide arose from the lateral wall of the orbit at the point of insertion of the lateral horn of the aponeurosis of the levator. This became tendinous to blend with the under surface of the aponeurosis. It then continued downward and forward to the medial side of the aponeurosis, paralleling the medial

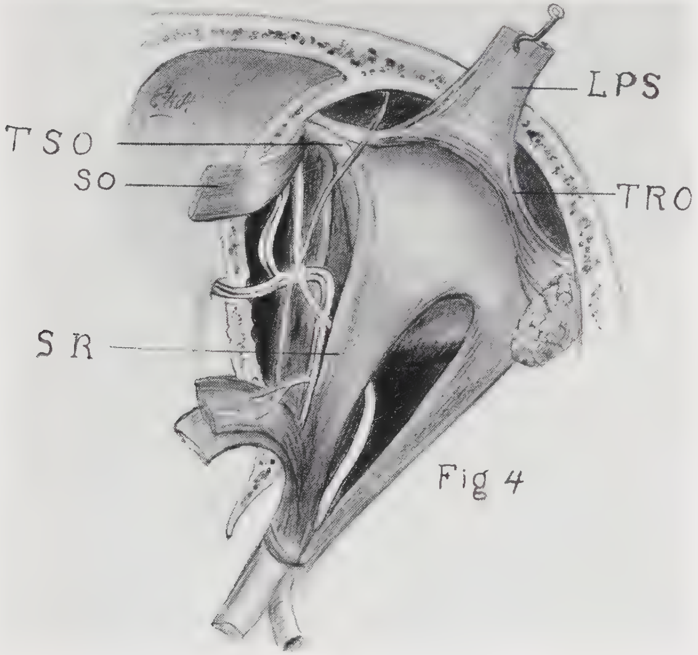

Fig. 4 Right orbit with the levator palpebrae superioris reflected to show *Tr.O.*, transverse orbital; *T.S.O.*, tendon of superior oblique to superior rectus.

cornua and inserting into the medial wall of the orbit, in common with the medial horn. In general this band paralleled the posterior convexity of the aponeurosis. When traction was made on the levator palpebrae superioris, this acted as a check ligament, preventing overelevation of the lid.

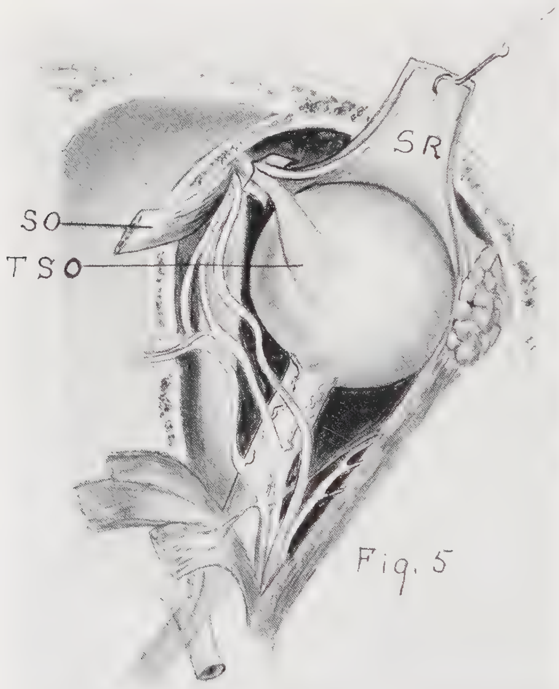

Fig. 5 Same orbit with the superior rectus reflected to show *T.S.O.*, a double insertion of the tendon of the superior oblique; *S.R.*, superior rectus.

SUPERIOR OBLIQUE

Reported variations of this muscle are few. LeDouble ('97) refers to one which he questions, reported by Colombo. Whitnall ('21) reported a double muscle; Morgagni, a fusion with the trochlea. Complete absence has been reported by a number of clinicians; LeDouble ('04), accessory slips.

Two cases of double insertion have been found in our laboratory: one in the right orbit of the female cadaver discussed above; the other (observed in 1920), in the left eye of an adult male cadaver.

Figures 4 and 5 are from the right eye of the female cadaver. In this case the tendon of the superior oblique (*T.S.O.*), as it passed through its pulley, divided almost equally, one part passed to the usual insertion in the sclera, the other into the medial edge of the superior rectus. This is not to be confused with the fascial sheath normally found passing from the pulley and reflected tendon to this muscle. Nor was it related in any way to the various reflections described by Sutton ('20).

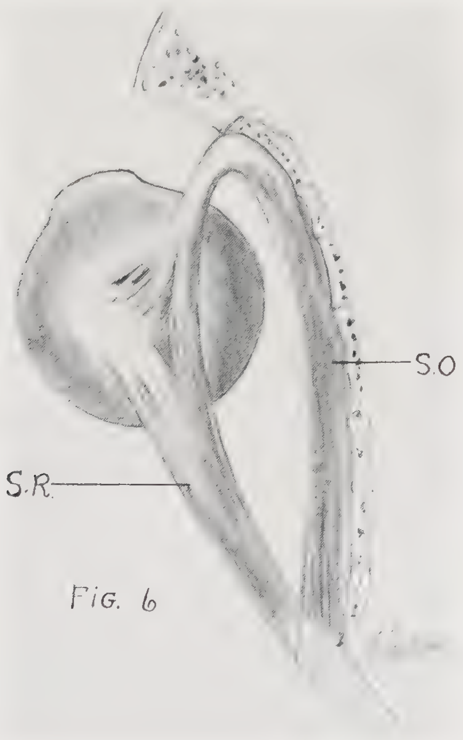

Fig. 6 A left orbit showing insertion of superior oblique.

Figure 6 was observed and sketched by Dr. H. J. Prentiss in 1920. In this case no part of the reflected tendon passed beneath the superior rectus to insert into the sclera. Excepting for a small falciform reflection that passed to the sclera medial to the insertion of the superior rectus, all the reflected tendon inserted into the superior surface of the superior rectus muscle.

Anomalies of these muscles have been explained by a faulty cleavage of the premuscular mesoblastic mass. In the case of the retractor of the fornix described above, we must consider, on this basis, a complete additional cleavage. Despite this additional layer, there was no reduction in the volume of either the usual levator palpebrae superioris or of the superior rectus.

In conclusion, appreciation is expressed to Mrs. Esther H. Powers for her careful sketches of these dissections.

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STUDIES OF AMPHIBIAN NEOTENY

III. THE GOLGI APPARATUS OF THYROID CELLS OF RANA CLAMITANS IN RELATION TO THE ANTERIOR PITUITARY

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ONE PLATE (TEN FIGURES)

There is considerable agreement in the literature as to the histological picture of activity of the thyroid gland. The most recent descriptions are those of Uhlenhuth ('27), Hirschlerowa ('27), Ingram ('29), dealing with amphibian material; and of Rabinovitch ('29) and Loeb and Bassett ('29), with mammals; all of these point to an increased height of the follicular epithelium and a loss of colloid from the follicles with the onset of activity. Bensley ('16), however, does not believe the amount of colloid present to be indicative of the active state. It has been pointed out repeatedly that the experimental picture is practically identical with that of the human thyroid in exophthalmic goiter, in which condition there can be little doubt as to the increased activity of the gland, although there are conflicting ideas as to the normalcy of the secretion produced and the underlying causal factors. Several interesting methods of stimulating the thyroid have been tried. It is usually little affected by drugs, and Uhlenhuth ('27) reports the failure of this method with amphibians. By exposing rats to cold for considerable periods, Cramer and Ludford ('26) found increased activity of the thyroid. Summarizing the results of a wide variety of experiments by different investigators upon amphibian metamorphosis—a process known to be related to the thyroid—and following his idea of a 'releasing mechanism' for the gland, Uhlenhuth was successful in utilizing extracts of the anterior pituitary with urodele material. Ingram ('29), by the method of transplantation, achieved similar results with anurans. Recently Loeb and his coworkers have also used extracts of anterior lobe to produce a marked state of activity in guinea-pig thyroid.

The reports of these workers are in general agreement, despite the wide variance in types of animal material used. Exposure to cold, however, is obviously unsuited for work with amphibians. The writer has found that lowering the temperature will greatly retard, or even prevent, metamorphosis as a result of anterior-lobe implantation. Activation of the thyroid by such implantation produces an artificial metamorphosis which most closely resembles the normal in all its stages. This is certainly not true for metamorphosis induced by administration of thyroid or iodine-containing substances.

To carry further his study of the changes induced in the thyroid by anterior-lobe treatment, the writer has wished to observe the so-called Golgi apparatus under such conditions. This interesting structure in its relationship to secretion has been discussed by a long list of investigators, and convincing proofs of its secretory function have been advanced by Nassonov ('23, '26, '27) and Bowen ('24, '26, '29). The integrity of the Golgi apparatus as a distinct structure has been attacked by Parat ('28), Owens and Bensley ('29), and others, who believe it to be an artefact produced by the reduction of osmic acid among the neutral-red-staining vacuoles of the so-called vacuome. However, Beams ('30) has recently presented evidence that the Golgi apparatus and the vacuome are separate substances occupying the same general region in the cell. The general literature has been extensively reviewed by Bowen ('29). In the specific case of the thyroid we do not have so much material. Bensley ('16) described changes in a cytoplasmic canalicular system in cells of the opossum thyroid which led him to believe that the normal mode of secretion is directly into the capillaries rather than into the follicular lumen. Cowdry ('22) observed similar changes in position of the Golgi apparatus in the guinea-pig thyroid, and he also suggests a reversibility of secretory polarity. Amphibian material is especially scant. Hirschlerowa ('27) found the apparatus considerably enlarged in the thyroids of normally metamorphosing Rana esculenta larvae, but was unable to show any tendency toward reversed polarity. Charipper ('29) describes variations in condition of the Golgi apparatus in Necturus.

Enlargement of the Golgi apparatus in thyroid activity is in accord with the observations of other workers using different tissues.[1] Ludford and Cramer ('28) found such an elaboration of the Golgi material in the rat thyroid following the cold treatment, in a case of exophthalmic goiter in the mouse, and in human exophthalmic goiter. While they occasionally found reversals in polarity of the Golgi apparatus following exposure to cold, they could find no evidence of reversed secretion; but in the mouse goiter reversals in position of the apparatus were frequent and in such cells the direction of excretion was toward the capillaries. Their failure to find the latter situation in the human they ascribe to iodine treatment.

The previous papers of this series dealt with the action of inorganic iodine in the metamorphosis of the Colorado axolotl and with the interrelation of thyroid and pituitary in Rana clamitans, a form which is partially neotenous in that the larval period is greatly prolonged beyond that usual for Anura. The present paper carries the observations upon the latter form to the Golgi apparatus of the thyroid cells and its behavior under the influence of the anterior pituitary.

MATERIAL AND METHODS

The tadpoles used in these experiments were of good average size, total length 70 to 90 mm., and hind-limb length 3 to 10 mm. The method of anterior-lobe transplantation previously described (Ingram, '29) was used, the pituitary tissue being taken from adult Rana pipiens and implanted intraperitoneally in the larval hosts. In most cases two transplants were made, at an interval of about a week, chiefly to avoid the possibility of loss or resorption. The majority of control animals received no treatment, many previous experiments having shown that operation and implantation of bits of muscle do not affect the thyroid. The animals were kept in lots of ten in glass aquaria. It is important that the water be changed frequently and that dirt and fecal matter be removed from the tanks daily, since tadpoles are quite susceptible to infections. The food consisted of algae, grated spinach, and small quantities of cooked liver.

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[1] Compare Da Fano ('22), Nassonov ('24), Brambell ('25), Weatherford ('29).

As the growth and metamorphic changes resulting from the transplantation progressed, individuals were killed at various stages and the thyroids removed for examination. Preparations for the Golgi material were made by the Weigl and Kolatchev-Nassonov methods, chiefly the latter. It was the experience of the writer that following fixation in Champy, exposure to 2 per cent osmic acid at 35°C. for three to four days was best for this material. Thyroids from control animals received the same treatment. In some cases the preparations were counterstained with acid fuchsin, in others no counterstain was used.

Anuran thyroid tissues are technically difficult under any conditions and great care must be used in handling the material in order to avoid injury. The normal, more inactive glands are especially difficult, being extremely delicate and susceptible to shrinkage, but the active glands, taken after the onset of metamorphic changes, were found to be much easier to prepare for cytological examination.

DESCRIPTION

Following the anterior-lobe treatment the usual general changes were found in the thyroids showing greatly increased activity. These changes have been shown to occur rather soon after treatment—within eight or ten days (Ingram, '29). Tadpoles thus treated during the winter respond more quickly than those experimented with in the spring, provided the water is kept reasonably close to optimum temperature. This probably depends upon the state of the donors' pituitaries prior to the breeding season, though further work is necessary to fix definitely the cause.

In the inactive thyroids of the controls the Golgi apparatus is rather compact, small, and delicate (figs. 1 and 2). Its shape is, of course, varied, displaying but slight tendency toward ramification, and is often reduced to a single curved filament. Ordinarily it hugs the nucleus closely and is situated between the nucleus and the lumen of the follicle, but in cases where the cells are greatly flattened, and even in more cubical cells, it may be found in a lateral position. In these cells the cytoplasm is relatively scant, especially in those bordering large, full follicles, and the size of the Golgi apparatus seems to conform to the amount of cytoplasm. Granular material is not abundant, although there must be some secretory activity by these cells, as evidenced by the accumulation of stored colloid and the appearance of occasional chromophobe colloid droplets at the apical margins of the cells. Probably such activity as occurs is periodic secretion by isolated cells and limited areas in the various follicles. True reversals in polarity of the Golgi material are rare, and those which do appear seem to have resulted from distortion of the cells under pressure of the follicular colloid.

A remarkable difference is found upon turning to the thyroids of an animal killed nine days after transplantation and in which the hind limbs have grown to a length of 24 mm. Here activity is at its height (figs. 5 to 10). The cells are elongate, and contain secretion droplets and blackened or grayish granules of various sizes, especially abundant in the apical region. The Golgi apparatus is strikingly large and complex, forming an intricate network and ramification which occupies a large part of the cytoplasm on the apical side of the nucleus. It conforms more or less to the shape of the cell in its distribution and relationships—it may be long and narrow in long and narrow cells, while in more cubical cells it may be flattened and spread out. In the latter type it is most likely to have basally directed portions extending down the side of the nucleus, or to be displaced laterally. Droplets of secretion may be seen in close relationship to the Golgi material, these being of either the staining or non-staining types, and sometimes conspicuous by virtue of their size, as may be seen from the figures presented (figs. 5, 7, 9, 10). There may also be more diffuse areas of stained colloid in the apical regions.

Examination of cross-sections of cells, and of sections cutting follicles tangentially, seems to show that the Golgi network usually fits against the nucleus as a sort of cap (fig. 8). True reversals of its position were extremely rare, very few being found in the careful examination of a large number of specimens. Certain areas of a section may give the appearance of reversals, but closer observation shows this to be due to tangential cutting. Sometimes the nucleus may be situated well toward the apex of the cell, but in such cases the Golgi material is still more apical in position. Occasionally the main body of the Golgi apparatus may be basal, but extensions toward the apex are found, these extensions being related to secretion droplets and secretion taking place in the normal direction—toward the follicular lumen (fig. 9). No evidence of reversed excretion was found in cases of reversal of the Golgi apparatus.

Material taken from animals killed after a longer interval from transplantation (about three weeks, when the animals were far advanced beyond the onset of metamorphosis and on the verge of the adult state) often shows involutional changes. Here the cells have fewer signs of activity, and colloid is again being stored in the follicles. The secretion process has evidently slowed down, as evidenced by diminution in intracellular secretory material. The Golgi apparatus (figs. 3 and 4) is smaller, and may give the appearance of fine curved rods, closely pressed against the nucleus. There is greater irregularity of position, with numerous lateral displacements. Frequent signs were found of recent extrusion of chromophobe colloid droplets, and in these cases it was interesting to find large numbers of black granules in the cytoplasm, the Golgi apparatus being poorly differentiated, although in the remainder of the section its structure was clearly demonstrated.

It would seem that the size of the Golgi apparatus is in a way proportional to the cytoplasmic area and the size and secretory content of the cells. In flat, inactive cells it is small, and apical or lateral in position; in active cells it is large, closely associated with secretory products, and apical in position in all but a few cases. In glands undergoing involution the apparatus may vary more in position, with a fair extent of spread and many variations in shape. In these latter preparations the Golgi material is in filaments, usually of good length, often knotted or looped, but sometimes almost straight.

DISCUSSION

That the anterior pituitary is clearly the controlling factor in thyroid activity as related to amphibian metamorphosis has been shown as discussed above and by the method of extirpation developed by Allen ('16) and Smith ('16). It has been suggested that the neotenous state depends upon some breakdown of the relationship between the two glands, which perhaps in turn depends upon some further controlling factor, as yet unknown. The material herein presented furnishes further proof that activity of the thyroid in these forms is incited by the anterior pituitary, since the relation of the Golgi apparatus to secretion has been demonstrated by Nassonov, Bowen, and others. It is perhaps not necessary to assume that the apparatus forms the secretion within itself, although Ludford and Cramer ('27) have thought this to be the case, but it is more likely that the materials for secretion are presented by the other portions of the cell, as suggested by Bowen, and merely further elaborated, collected, and differentiated at the surfaces of the Golgi material. Increased surface would then be necessary with increased activity. Certain peculiarities in form have led Bowen to suggest that the material exists in plate-like formations which in section resemble threads. The appearance in many tadpole thyroid cells bears out this supposition.

Whether or not the anterior-lobe hormone acts directly upon the cytoplasm of the cells is an interesting question. It may well be that the action is more in the nature of a 'release,' in which, according to Uhlenhuth ('27) and Hirschlerowa ('27), intercellular canals are opened, furnishing a pathway from the follicular lumen to the capillaries. As a response to the resultant loss of colloid from the follicles the cells may hypertrophy and enter upon a state of increased activity, for the purpose of replacing the lost colloid. However, the hypertrophy of the cells and loss of colloid go together, so far as the writer can determine, somewhat increased height of the epithelium having been observed before a marked loss of colloid was evident. The stimulus is apparently directly upon the cells, with the result as expressed by changes in the Golgi apparatus described above, provided one rules out a nervous intermediary which is not improbable, though denied by Uhlenhuth ('27).

The resemblance between these active glands and those in human exophthalmic goiter is curious, especially since some medical investigators believe there is a possibility of diminished activity of the gland in this disease, or that there may be an abnormal secretion formed. This latter suggestion is emphasized by Ludford and Cramer ('28), who point out the apparently reversed direction of secretory output in some cells of a case of exophthalmic goiter in the mouse. Their observations upon similar human material show some reversal in position of the Golgi apparatus, but they were unable to demonstrate clearly an extrusion of secretory material directly toward the capillaries—which failure they ascribe to preoperative treatment with iodine. They then postulate the production of an abnormal type of secretion by this method. It is difficult to understand why activated glands in amphibians, whose activity is also manifested by emaciation, exophthalmia, nervousness, and weakness, should not show the same sort of thing, although the writer admits that it is a long step from a tadpole to even a mouse. The whole question of reversal of polarity of the Golgi material in gland cells is somewhat puzzling. A number of investigators have noted such displacements (compare Courrier and Reiss, '22; Cowdry, '22; Jasswoin, '24; Bowen, '26; Turchini, '27; Giroud, '28), and Cowdry has suggested that in the thyroid, excretion might normally take place directly into the capillaries. It has also been suggested that this condition may be due to mechanical factors. Bowen and others cite a number of instances of reversals without evidence of change in excretory polarity. Ludford and Cramer, as discussed, support Cowdry's suggestion for special cases. The present writer cannot confirm this for metamorphosing amphibians which present much the same general picture, histologically and in gross, if one considers the exceedingly scant number of reversals or the failure to find indications of a change in secretory polarity. Interesting evidence from mammalian material could perhaps be obtained by the methods of Loeb and his coworkers, using anterior-pituitary extracts with guinea-pigs. Probably the direction of excretion depends upon physicochemical factors, including the problem of membrane permeability and related polarity.

Uhlenhuth ('27) describes the appearance of chromophobe colloid vacuoles in the basal portions of the cells in salamander thyroids and their subsequent migration to, and extrusion from, the apical cell end. The writer has been unsuccessful in confirming this, except for a few cases in which colorless vacuoles could be seen rather low down in the cell. In view of the Nassonov theory, which calls for the formation of secretory material in close relationship with the Golgi apparatus, Uhlenhuth's observations seem hard to explain. Can it be that these droplets are formed without benefit of the Golgi material? There is the possibility that they may have been formed in connection with basally projecting extensions from the apparatus, since in active thyroid cells the Golgi material ramifies widely. In the early stages of secretion, when the cells are small, formation of secretion droplets basally might well be carried on by the Golgi apparatus in one of its varying positions without necessitating a reversal of polarity. Such ramifications as are described above are common in glands of the serous type.

It is interesting to inquire which of the several hormones ascribed to the anterior pituitary causes the effects described. The evidence available here is in favor of the growth principle, since the writer has examined material from numerous individuals and has found no precocious sexual development following implantation with anterior pituitary, within the time limits of the experiments with anurans. Burns ('30), however, reports the onset of sexual maturity following such treatment in salamanders. Loeb's group finds no marked sexual effects by their extract which stimulates the thyroid, while transplantation methods which do not affect the thyroid will promote sexual maturity in mammals. It is odd that the two methods of approach should give directly opposite results in the different classes. More precise evidence can only be obtained by further work, which is in progress.

SUMMARY

1. The method of transplantation of anterior pituitary has been used in inducing greatly increased thyroid activity and metamorphosis in Rana clamitans, and the behavior of the Golgi apparatus in the thyroid cells observed.

2. In small, relatively inactive thyroids the Golgi apparatus is small and delicate, a simple network or looped filament, usually situated close to the apical pole of the nucleus. This location is subject to variation, and lateral displacements are not uncommon, although reversals in polarity are very rare.

3. In activated thyroids the Golgi apparatus develops in proportion to the size of the cells, forming a very large, complex network, with ramifications extending throughout the apical portion of the cell, in close relationship with secretory droplets. Reversals in position are very infrequent,

and in such cases no evidence of reversed secretory polarity was seen, the products still being produced in the direction of the follicular lumen.

4. In involutional stages the size and extent of the Golgi apparatus are reduced and its position subject to greater variation. The amount of Golgi material is proportional to the area of the cytoplasm and the amount of secretory material it contains.

5. The relationship of the material presented to various theories of the mechanism of thyroid secretion is discussed.

6. Evidence is presented which suggests that the growth principle of the anterior pituitary, apart from the sex-maturity principle, is the controlling factor for enhanced thyroid activity and amphibian metamorphosis.

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PLATE 1

EXPLANATION OF FIGURES

The figures were drawn from sections 4 to 5 μ thick, with the aid of a camera lucida and a B. & L. 2-mm. objective with 10× ocular, with the exception of figure 7, for which a 4-mm. objective with 10× ocular was used. The Golgi apparatus is deep black. Nucleus is outlined, but otherwise unshaded. *C.*, colloid; *S.*, secretion droplets and vacuoles; *N.*, nucleus.

1 Cells from the inactive thyroid of an immature tadpole, showing variations in the Golgi apparatus.

2 Same as figure 1, but larger cells.

3 Cells from a thyroid which is undergoing involution.

4 Same as figure 3, but smaller cells.

5 Cell from active thyroid, showing a large secretion droplet.

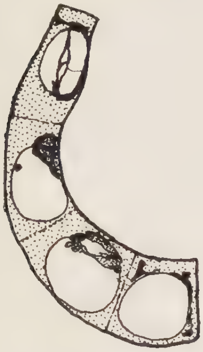
6 Cell from active thyroid, showing extent of Golgi apparatus.

7 Entire follicle from thyroid of a tadpole undergoing metamorphosis, showing the uniform activity of the cells, and large Golgi apparatuses, with intracellular secretion vacuoles and droplets. Note the small follicular lumen.

8 Cross-sections of cells from an active thyroid, showing the extent of the Golgi apparatus laterally and its close contact with the nucleus.

9 Cells from an active thyroid. Middle cell shows reversal in position of the Golgi apparatus, with apical extension related to secretory material.

10 Cells from an active thyroid, showing a large droplet of chromophobe colloid and variations in the Golgi apparatus.



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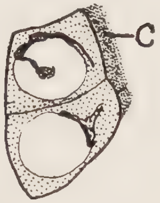

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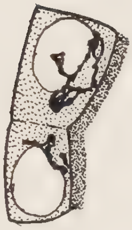

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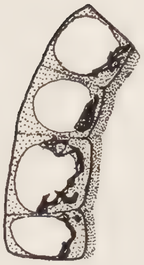

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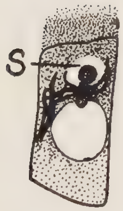

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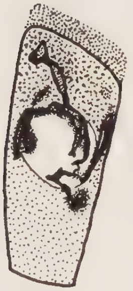

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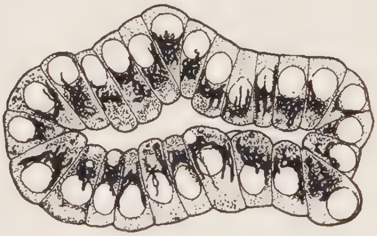

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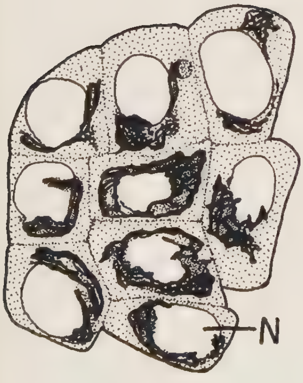

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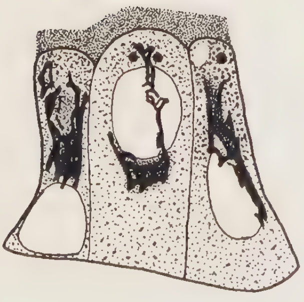

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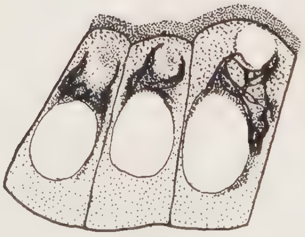

10

AN HEREDITARY ANTERIOR-PITUITARY DEFICIENCY IN THE MOUSE

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THREE FIGURES

The importance and interest of determining the factor responsible for the dwarfism, infantilism, and other disabilities which appear spontaneously in a strain of mice, reared by one of us at Cold Spring Harbor, caused us to study in this unique material the growth rate, the structure of the endocrine organs, and, later, to institute an endocrine therapy in an endeavor to determine physiologically the primary cause of the various disabilities present. These studies, which can now be reported upon in a preliminary paper, strongly indicate that the primary causal factor of the dwarfism and associated disabilities is an anterior-pituitary deficiency, for the endocrine disabilities simulate those displayed by hypophysectomized rats (Smith, '27, '30) and, as in the case of the hypophysectomized rats, daily implants of fresh anterior lobe bring these defective animals to a normal condition.

By surgical removal of the anterior lobe of the pituitary in young rats and subsequent replacement therapy, it has been shown that the dwarfism and endocrine regression following the operation are directly due to the absence of pituitary secretions. The discovery, reported in this paper, that hereditary dwarfism in the mouse is associated with the absence of eosinophiles in the anterior lobe of the pituitary and with deficiencies of other endocrines closely resembling those of the hypophysectomized rat gives considerable support to the hypothesis that the defect of the anterior lobe found in the dwarf mouse was the common cause of its various other characteristics. The accuracy of this hypothesis we have demonstrated by subjecting dwarf mice to daily transplants of fresh anterior lobe.

The hereditary dwarfism has been found in a strain of black silver mice brought to this country from England by Prof. L. C. Dunn. Snell ('29) has published his findings with breeding records (103 normal, 34 dwarfs from matings giving any dwarfs) and concludes that the dwarfism behaves in inheritance as a recessive mendelian character depending upon a single gene. The animals herein described came from an uncrossed, inbred branch of this strain which started from a pair contributed to the Department of Genetics by Professor Dunn in 1928.

Snell has described the general features of the dwarf mouse; dwarfism is not manifest until the end of the second week, when the natural process of weaning normally begins with the first eating of solid food; after this, development and growth in the dwarf practically cease, leaving the mouse in an infantile condition. Snell has personally demonstrated to the authors a microscopic preparation of the defective thyroid of a dwarf.

We have studied the endocrine glands of six dwarfs, the finding having been harmonious throughout the series. The thyroids are extremely reduced in size and the glandular tissue is usually not compact, but separated by adipose and connective tissue. The thyroid tissue is, therefore, not aggregated into a discrete organ, thus making its accurate dissection and weighing impossible. We found it wise to section the entire larynx and surrounding tissue. Some of the thyroid tissue is not organized into follicles and contains little or no colloid. The vesicles which are present are lined by a squamous epithelium with flattened nuclei, contrasting with the cuboidal type present in the normal controls. The adrenal cortex is reduced in thickness and the characteristic zonation is absent or indistinct. The medullary cells appear to be normal and contain the usual proportion of cells showing the chromaffin reaction.

The gonads, although clearly much delayed in development, do not show as profound an aplasia as we had anticipated would be present, from studies upon hypophysectomized rats. In the ovary, although no medium-sized or large follicles or corpora lutea are present, development has proceeded to a stage beyond that ever occurring after the complete ablation of the anterior lobe. In the testes and other parts of the reproductive system of the male, likewise, development appears only to be delayed. In the seminiferous tubules spermatocytes are present and undergoing division, and in occasional tubules spermatids are fairly numerous. The testes are not flabby as in hypophysectomized rats.

The defect in the pituitary appears to be limited to the anterior lobe. Although we have not carried out quantitative studies and our observations must be checked later by measurements, it now appears that the posterior lobe (neural and intermediate lobes) is not subnormal in size. Structurally, no abnormalities have been observed in it. The anterior lobe, however, is markedly abnormal. No eosinophiles are present. In section this lobe has the appearance of a connective-tissue network, which may be either compact or loose. Although we are certain about the absence of the eosinophiles, we cannot speak with equal certainty concerning the presence or absence of basophiles. All the pituitaries were sectioned serially at 3 μ and parts of each stained with Mallory's connective-tissue stain, with Altmann's acid fuchsin and acid violet according to the technique given by Bailey, and with Masson's trichrome stain. These stains, which are adequate in other forms upon which we have worked, have not stained satisfactorily the basophiles in our normal mice, so that we are unable to state whether in the dwarfs basophiles are present or not. Chromophobe cells, if present, are much reduced in number.

Daily implants of fresh rat anterior lobe into a series of six mouse dwarfs have been made, using the same technique as previously described by one of us, except that the transplants were placed alternately in the hind legs instead of all four.

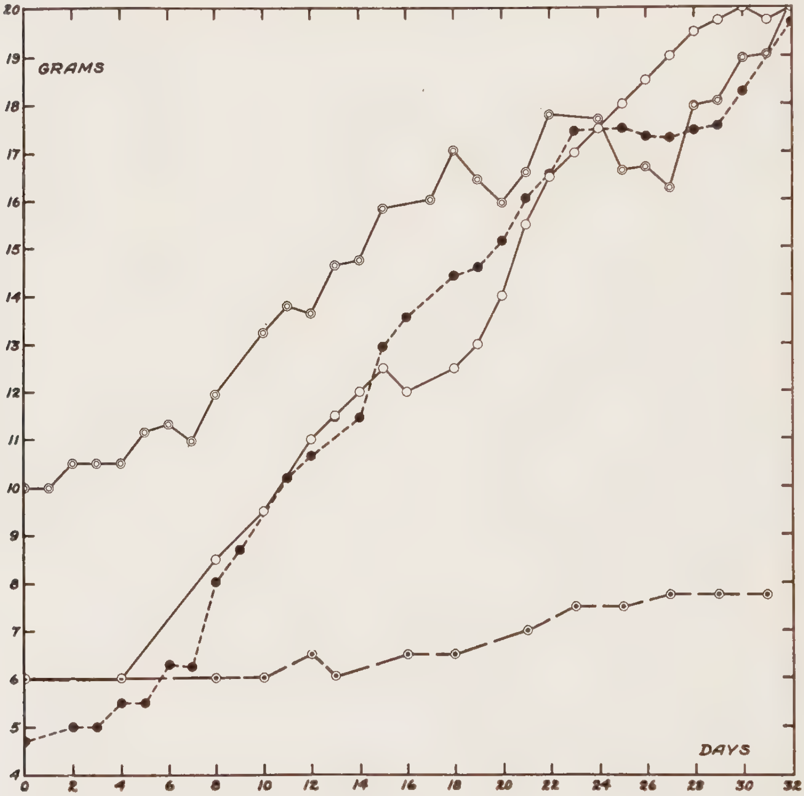

Fig. 1 Body weights, over a period of thirty-two days, of three male dwarfs treated daily with transplants of fresh anterior lobe of rat. Open circles, ♂ 65054, treatment started at thirty-one days; circles with dot, ♂ 65056, litter mate of preceding, lower curve before treatment, upper curve during treatment, which began at 106 days; solid points, ♂ 67187, treatment begun at thirty days.

In every case the treatment has given positive results. The response in terms of body weight did not appear until after four to seven days, when a marked rise in daily weight increments from practically zero to approximately half a gram a day was found (figs. 1, 2). Although the dwarfs readily withstand the anesthesia used in making the daily transplants, one died on the sixth day of treatment, not having yet shown any advance in body weight. The organ weights of this mouse and the histological study of its glands showed that the endocrine restoration had made considerable progress. In the other five cases, along with the striking resumption of growth, came a rapid loss of the dwarf traits. In place of docile inertia appeared the usual exaggerated

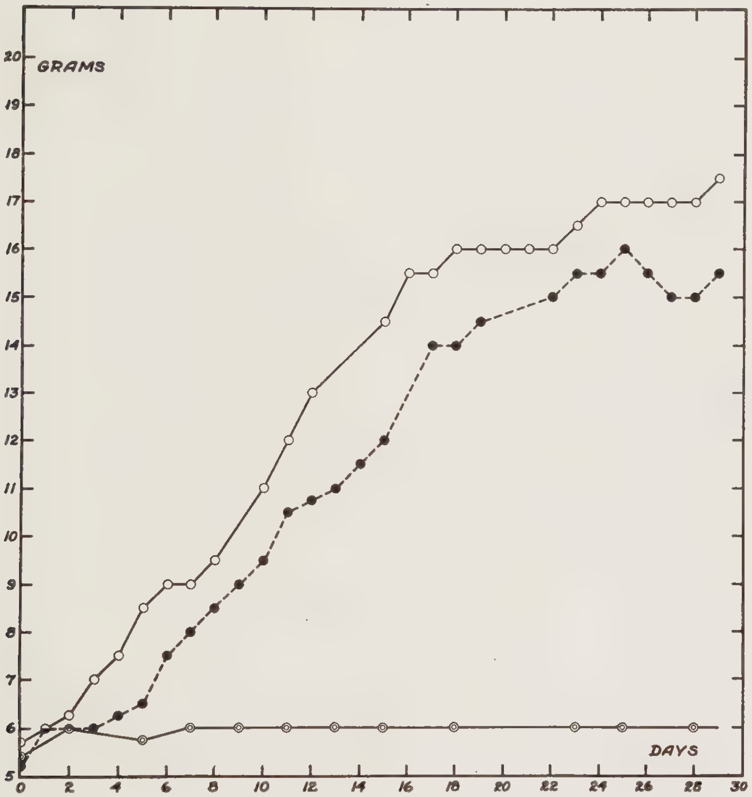

Fig. 2 Body weights, over a period of thirty days, of two female dwarfs treated with fresh rat anterior lobes, and one untreated dwarf; open circles, ♀ 65671, treatment begun at thirty-six days; solid points, ♀ 65518, treatment begun at thirty-four days; double circles, ♀ 65517, no treatment, litter mate of preceding mouse.

dynamics of the growing young mouse; the external genitalia became normal; the head assumed normal proportions; externally, the dwarfs could not be distinguished from normal mice (fig. 3). But even the dwarf treated the longest did not equal the normal litter mate in body weight; one male dwarf treated over a period of seventy-six days reached 25 grams, whereas its litter mate at the same age weighed 35 grams. This is a matter of adiposity and not merely skeletal size. Such comparisons do not have special interest, since even in normal mice differences in weight at weaning due to differences in amount of mother's milk may not be made up for more than two months afterward (MacDowell, unpublished). The differences between the normal and the dwarf mice at the beginning of treatment are twice as great as in the normal and overfed series mentioned above.

Besides eliminating the external dwarf characteristics, the treatment has brought these mice to sexual maturity. The first treated male dwarf mated with a normal female on the twenty-third day of treatment, having reached the weight of 17.5 grams and the age of fifty-five days. Five litters sired by this dwarf have been born, giving a total of thirty offspring. In the treated female the vagina opens in the six- to eight-day period characteristic of the treated rats, hypophysectomized before maturity. Two females that had reached 18 grams after thirty and forty treatments, respectively, showed ovaries with many corpora lutea and with some large, though abnormal, follicles. Vaginal smears gave evidence of irregular oestrous cycles.

That transplants of anterior pituitary make the dwarfs grow up and become sexually mature gives presumptive evidence that the endocrine deficiencies have been restored. Studies of the gross and sectioned material show that such is the case. Table 1 gives the organ weights for the treated and untreated dwarfs. Too much reliance should not be placed on the matter of organ weight, especially in such small numbers, since many variables are involved besides the one of immediate interest. The histological differences, how-

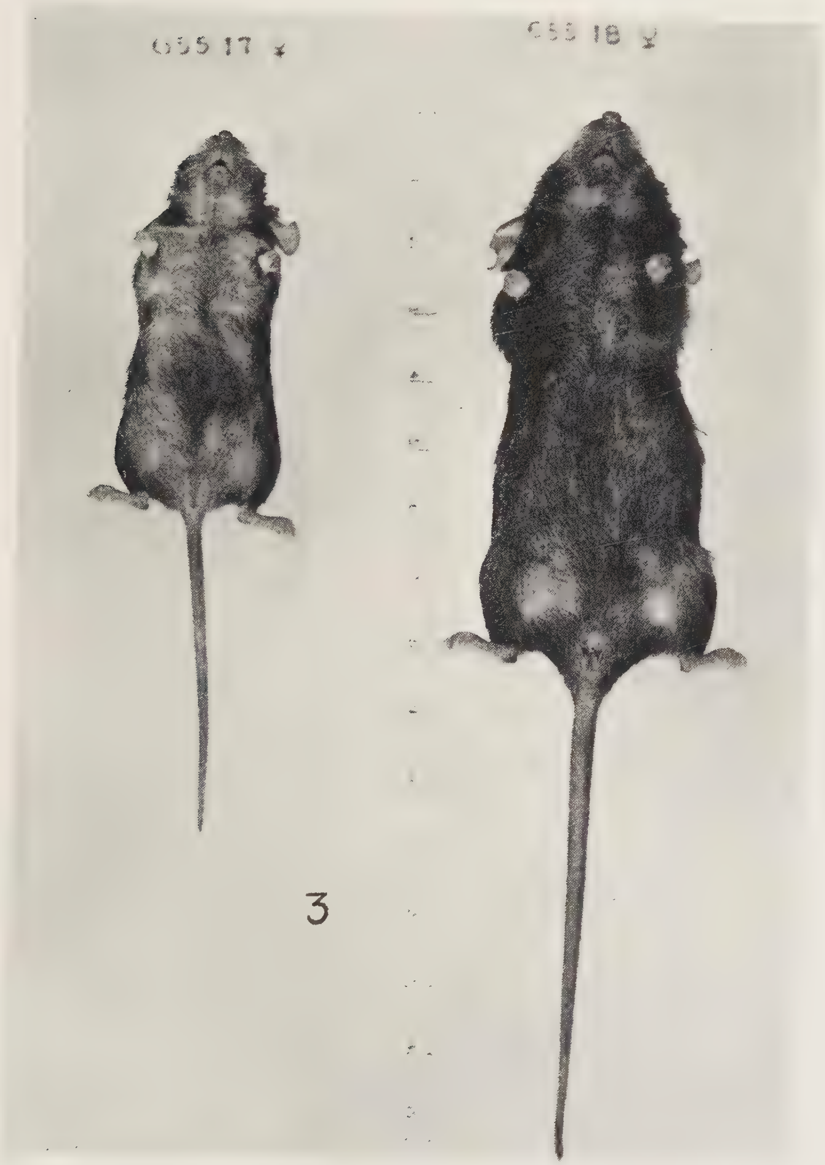

Fig. 3 Photographs of a treated dwarf and an untreated dwarf litter mate at the time of autopsy. Both were autopsied at the age of eighty-four days, forty implants of fresh anterior lobe over a period of fifty days having been given to 65518.

TABLE 1

Body and organ weights in grams of normal and treated dwarfs. Consecutive pedigree numbers indicate litter mates. All mice from a highly inbred strain of silvers

MOUSE	NUMBER OF TRANS-PLANTS	BODY WEIGHT		ABSOLUTE WEIGHTS				PER CENT OF BODY WEIGHT			
		Beginning	End	Gonads	Genital system less gonads[1]	Adrenals	Hypophysis	Gonads	Genital system less gonads[1]	Adrenals	Hypophysis
♂ 65052 normal	0	18	35	.1951	.841	.0034		.557	2.403	.010	
♂ 65056 dwarf	0	6	10	.0882[2]				.882[2]			
♂ 65054 dwarf	62	6	25.5	.2150[3]				.843[3]			
♂ 65056 dwarf	30	10	20.1	.1780[4]	.664	.0040	.0009	.886[4]	3.303	.020	.004
♂ 67187 dwarf	34	4.7	22.2	.1569	.4697	.0041	.0006	.707	2.116	.018	.003
♀ 63421 normal	0		16.2	.0080	.109	.0053	.0011	.049	.673	.033	.007
♀ 64889 normal	0		22.0	.0103	.0836	.0072	.0018	.047	.380	.033	.008
♀ 64888 dwarf	0		6	.0033	.0082	.0010		.055	.137	.017	
♀ 63422 dwarf	0		4	.0014	.0035	.0003	.0002	.035	.088	.008	.005
♀ 65055 dwarf	0		4	.0027	.0035	.0006[5]	.0001	.068	.088	.015[5]	.003
♀ 65057 dwarf	0		6	.0030	.0040	.0008	.0002	.050	.067	.013	.003
♀ 65517 dwarf	0		6.5	.0024	.0121	.0010	.0002	.037	.186	.015	.003
♀ 65518 dwarf	40	5.2	18.5	.0585	.0682	.0052	.0005	.316	.369	.028	.003
♀ 65671 dwarf	30	5.7	17.5	.0498	.1078	.0053	.0004	.285	.616	.030	.002
♀ 65053 dwarf	6	6	5	.0122	.044	.0019		.244	.880	.038	

[1] In male, includes bladder; in female, uterus and tubes.

[2] 2× weight of left testis removed at 106th day, when treatment began.

[3] 2× weight of left testis.

[4] 2× weight of right testis after treatment.

[5] 2× weight of right adrenal (left adrenal lost).

ever, are beyond all possible question. The adrenals, thyroids, and testes of the treated dwarfs cannot be distinguished from those of normal litter mates. One outstanding difference, however, still distinguishes the treated dwarfs from normal mice: the anterior lobe of the pituitary remains in the same defective condition as in the untreated dwarfs.

Dwarfism, sexual infantilism, and certain endocrine abnormalities are transmitted as a unit in inheritance; by the administration of the material of one of the glands, all the abnormalities are removed, with the exception of the defect of the gland administered. This gland, the anterior pituitary, has been shown previously to be necessary for normal development of the other glands and of the individual as a whole. The evidence seems conclusive that the developmental peculiarities of the dwarf mouse are due to a deficiency of the anterior pituitary gland, which is inherited in an alternative manner.

SUMMARY

By daily transplants of fresh rat pituitaries, the small size, sterility, and associated endocrine abnormalities of a hereditary dwarfism carried by a strain of silver mice have been corrected, with the outstanding exception of the deficiency in the anterior lobe of the pituitary. It is concluded that the deficiency of the anterior lobe is primarily causal to the other observed abnormalities of the dwarfs.

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A CASE OF CONGENITAL HERNIA THROUGH THE LEFT CENTRAL LINEA SEMILUNARIS OF THE DIAPHRAGM (CAT)

GEORGE M. PLAGENS AND JAMES T. BRADBURY[1]

FOUR TEXT FIGURES AND ONE PLATE (TWO FIGURES)

INTRODUCTION

A diaphragmatic hernia may be defined as a protrusion of a portion of the abdominal viscera into the cardiac or either of the pleural cavities through a rupture in the diaphragm. These hernias are of three types, congenital, acquired, and traumatic.

While hernias through the diaphragm are rare, they are not so rare as has been formerly supposed. They are frequently found in anatomical laboratories, and have been discovered radiographically in infants as well as in adults. Approximately 1200 cases in humans are on record, as reported by Dorland and Hubeny ('26). They have also been found in the lower mammals; in the Belgian hare, as reported by Thomson ('00), and in the cat, as reported by Brisco ('28). The condition is characterized by an escape of a portion of the abdominal viscera, as a rule, into the left pleural cavity. The extent of the involution varies greatly; in some cases only a portion of the stomach is concerned, while in other cases the greater portion of the abdominal viscera has escaped into the thoracic cavity. But, to our knowledge, there has been no case reported that involves as large a portion of the abdominal viscera as the one to be described in this paper.

The authors wish to express their appreciation to Dr. Peter Okkelberg for his assistance in the preparation of this paper.

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[1] Contribution from the Department of Zoölogy, University of Michigan, Ann Arbor, Michigan.

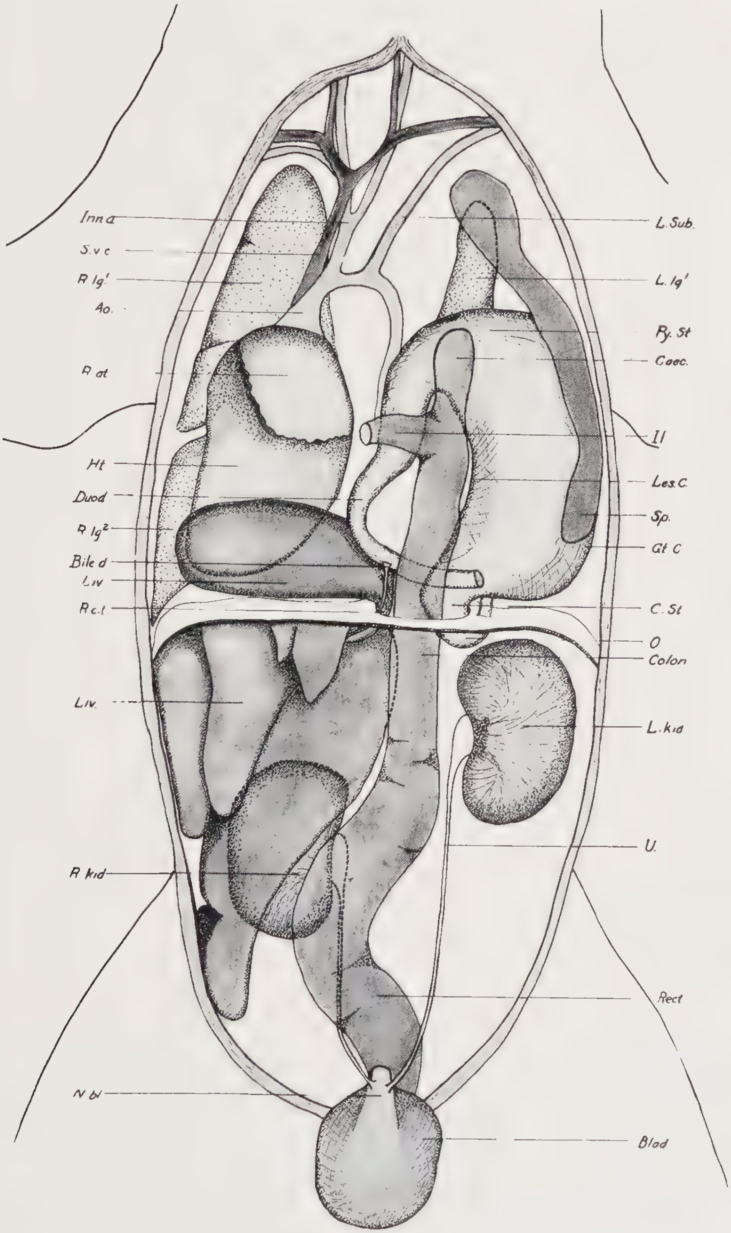
OBSERVATIONS

The specimen to be described was found among those used for dissection in the Comparative Anatomy Laboratory of the University of Michigan. It was a male cat of medium size and was apparently in good health when killed and prepared for laboratory study.

When the body cavity was opened for study of the viscera, it was found that the great omentum covered the organs in the thorax (fig. 5). Further examination showed that a large portion of the abdominal viscera had migrated into the thorax. The only part of the alimentary tract in the abdomen was the colon, extending directly through the diaphragm and abdominal cavity into the pelvis (fig. 1). The liver was greatly distorted and occupied a position in the right side of the abdomen. The left kidney lay immediately caudad of the diaphragm, but the right one was so far displaced that its position was 3 cm. caudad to that of the left. These positions are the reverse of the normal condition.

In the thorax the spleen was found to lie against the left dorsal wall (fig. 6). The small intestine filled most of the ventral thoracic space immediately above the omentum and extended forward into the apex of the left pleural cavity. The colon extended through the aperture in the diaphragm craniad, so that it formed a straight tube from the caecum to the rectum. The stomach (fig. 1), in passing through the rupture, had rotated through an angle of 180° from its normal position, so that its pyloric portion extended cephalad, its dorsal side ventrad, and its greater curvature against the left thoracic wall. The oesophagus entered the abdominal

Fig. 1 Diagram showing the distribution of the viscera in the abdomen and thorax. *Ao.*, aorta; *Blad.*, bladder; *Bile d.*, bile duct; *Cae.*, caecum; *C.St.*, cardiac stomach; *Duod.*, duodenum; *Gt.C.*, greater curvature of the stomach; *Ht.*, heart; *Inn.a.*, innominate artery; *Il.*, ileum; *Les.C.*, lesser curvature of the stomach; *Liv.*, liver; *L.kid.*, left kidney; *L.lg.*^{1}, left lung; *L.Sub.*, left subclavian artery; *N.bl.*, neck of bladder; *O.*, oesophagus; *Py.St.*, pyloric stomach; *R.at.*, right atrium; *R.c.t.*, ruptured central tendon; *R.ly.*^{1}, ^{2}, lobes of right lung; *R.kid.*, right kidney; *Rect.*, rectum; *Sp.*, spleen; *S.v.c.*, superior vena cava; *U.*, ureter.


Inn a
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R lg^1
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Duod
R lg^2
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N bl
L.Sub.
L. lg^1
Py. St
Caec.
Il
Les.C.
Sp.
Gt C
C. St
O
Colon
L.kid
U.
Rect
Blad

Figure 1

cavity through its hiatus and immediately made a U turn back through the rupture to the cardiac stomach. A lobe of the liver, probably the left lateral, had also migrated through the diaphragm and lay to the right of the stomach in contact with the mediastinum, with which it had formed an adhesion. The left lung (fig. 2) was composed, as normally, of three lobes, but they were about one-fourth the normal size and occupied a position against the dorsal thoracic wall. The right lung was two or three times the size of the left, but it was smaller than normal, due to the pressure of the viscera upon it. The viscera in the left pleural cavity had displaced the mediastinum so far to the right that the ventral portion of it was in contact with the ventrolateral

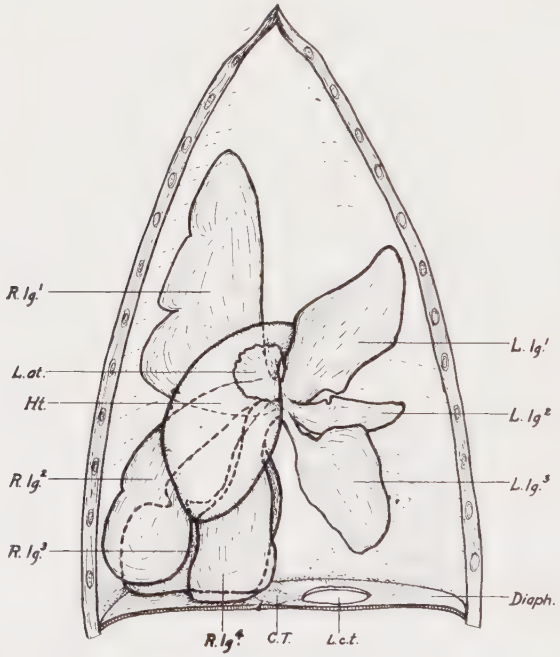

Fig. 2 Diagram showing the relative size of the lungs and the position of the heart in the thorax. *C.T.*, central tendon; *Diaph.*, diaphragm; *Ht.*, heart; *L.at.*, left atrium; *L.c.t.*, left central tendon, ruptured; *L.lg.*^{1}, ^{2}, ^{3}, lobes of left lung; *R.lg.*^{1}, ^{2}, ^{3}, ^{4}, lobes of right lung.

thoracic wall (fig. 3). This movement of the mediastinum had caused the right lung to assume a dorsolateral position. Caudad of the heart, however, the right lung expanded ventrally, so that it was in normal relationship with the pleural surface of the diaphragm.

The lateral movement of the mediastinal septum had carried the heart to the right to a position against the lateral thoracic wall (fig. 3). The dorsal portion of the septum had carried the descending aorta and oesophagus to the right of the vertebral column in the midthoracic region. The intercostal branches of the aorta crossed over the centra to the left to reach their position along the ribs.

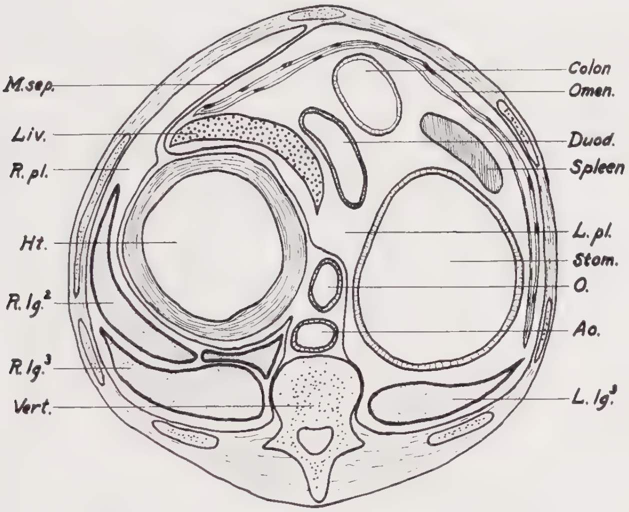

Fig. 3 A reconstructed cross-section through the thorax about 5 cm. above the diaphragm. Viewed from the caudal surface. The figure represents the relative distribution of the abdominal viscera in the thorax. The dorsal displacement was really much greater, since the small intestine has been purposely omitted. *Ao.,* aorta; *Col.,* colon; *Duod.,* duodenum; *Ht.,* heart; *Liv.,* liver (left lateral lobe); *L.lg.*^{3}, lobe of left lung; *L.pl.,* left pleural space; *M.sep.,* mediastinal septum; *O.,* oesophagus; *Omen.,* great omentum; *R.lg.*^{2}, ^{3}, lobes of right lung; *R.pl.,* right pleural space; *Sp.,* spleen; *St.,* stomach; *Vert.,* vertebra.

The pancreas and spleen, carried into the thorax by their connections with the mesenteries and omentum, seemed normal in every respect.

The viscera in the left pleural space had crowded the left side of the diaphragm so far caudad that it had lost its dome-like curvature. However, the right side of the diaphragm had maintained a more normal convexity. The muscular elements were lacking in the left half of the diaphragm, but the right side was muscular enough to have accomplished respiratory movements. The right half of the central tendon was very distinct and in contact with the margin of the rupture (fig. 4). The triangular membranous portions of the central tendon were distinct, the right one being longer than the left. Since the left one lay in contact with the dorsal margin of the hernial ring, it indicates clearly that the hernia occurred through the left half of the central tendon.

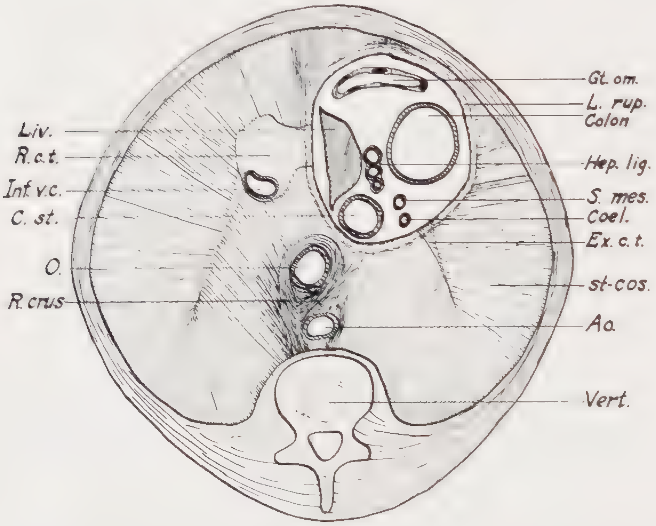

Fig. 4 A semidiagrammatic section showing the abdominal surface of the diaphragm. The figure shows the relative size of the hernial opening in the diaphragm and the relationships of the viscera passing through it. *Ao.*, aorta; *Coel.*, coeliac artery; *Colon*, colon; *C.st.*, cardiac stomach; *Ex.c.t.*, extension of central tendon (triangular membranous portion); *Gt.om.*, great omentum; *Hep.lig.*, hepatic ligament; *Inf.v.c.*, inferior vena cava; *Liv.*, liver (left lateral lobe); *L.rup.*, rupture in left central tendon; *O.*, oesophagus; *R.crus*, right crus; *R.c.t.*, right central tendon; *S.mes.*, superior mesenteric artery; *st-cos.*, sternocostal portion of diaphragm; *Vert.*, vertebra.

On the pleural surface of the diaphragm the line of attachment of the mediastinum was tangent to the right border of the aperture. On the peritoneal surface of the diaphragm the line of attachment of the coronary ligament continued through the right portion of the rupture onto the mediastinum.

The rearrangement of the mesenteries had brought about some interesting adjustments of the blood supply to the viscera. The hepatic artery came off from the coeliac axis below the diaphragm and entered the duodenohepatic ligament, where it divided immediately to give off the gastroduodenalis. The hepatic continued caudad into the liver. The gastroduodenalis passed forward in the duodenohepatic ligament together with the common bile duct and the hepatic portal vein. Normally the hepatic artery runs through the duodenohepatic ligament, but in this case it had been replaced by the gastroduodenalis. The coeliac, after giving off the hepatic, passed forward through the rupture in the diaphragm and there its lienal and right gastric branches were easily recognized.

The superior mesenteric passed forward through the aperture, where its branches, the middle colic, right colic, and ileocolic, were easily identified. The right colic, which normally supplies the ascending and transverse colon, supplied that portion of the colon lying in the passage through the diaphragm. It had an anastomosis craniad with the ileocolic. The middle colic doubled back along the superior mesenteric for a distance of 2 or 3 cm. from its origin and then continued caudad in the dorsal mesocolon. As it passed through the diaphragm it gave an anastomosing branch to the right colic. In the midabdominal region it passed from the mesocolon onto the dorsal surface of the colon, giving off a branch which anastomosed craniad with the right colic, and then continued caudad into the pelvis as the superior hemorrhoidal.

The inferior mesenteric was so small that it could be traced only as far as three minute branches in the mesocolon, none of which had any visible connections with the superior hemorrhoidal. Normally the inferior mesenteric passes onto the dorsal surface of the colon and gives rise to an anterior and a posterior branch; the anterior (left colic) anastomoses with the middle colic, and the posterior (superior hemorrhoidal) passes into the pelvis. In this cat the left colic and superior hemorrhoidals were merely continuations of the middle colic.

The hepatic portal system is normal except in that it drains caudad, whereas its normal direction of drainage is craniad.

DISCUSSION

According to Babcock ('29), one-sixth of the cases of diaphragmatic hernia are congenital, one-sixth acquired, and two-thirds due to trauma resulting from a penetrating rib, as the result of a crushed chest or the like, or from a rupture of the diaphragm by increased intra-abdominal pressure.

To what category this case belongs is difficult to determine. We are unable to find any indications that the animal had ever met with a serious accident. The skeleton of the chest appeared normal and no indications of broken bones were discovered. If any broken bone had punctured the diaphragm, it is highly improbable that it could have healed without leaving a scar or rib deformity. If the abdomen had been subjected to some great pressure, sufficient to rupture the diaphragm, it is reasonable to assume that the abdominal viscera would have suffered injuries sufficient to bring about the death of the cat. Further evidence points to an early migration of the viscera and favors an origin of the hernia *in utero*. The chances that this hernia was due to an accidental injury are very slight.

In the embryonic development of the diaphragm, the central tendon develops in the middle portion of the septum transversum, which forms very early as a membranous septum, dividing the primary body cavity into the thorax and abdomen. The musculature of the diaphragm has its origin in fibers from the centra of the vertebrae and from fibrous bundles which interdigitate with the transversus abdominalis.

The fibers converge and insert into the central tendon. Thus the central tendon remains as an irregular crescent-shaped membranous area in the central portion of the diaphragm. It seems possible that this membrane might have ruptured at some stage of embryonic development, owing to a contraction of the uterus or to other similar factors that might have forced the abdominal organs into the thorax.

This view seems to offer a more plausible explanation of the etiology of the hernia. It is substantiated by the following anatomical anomalies: 1) The musculature of the diaphragm had developed only on one side. The hernia had existed so long that if any muscle tissue had ever been present in the left half of the diaphragm it had completely atrophied. 2) The gastroduodenal artery had taken the place of the hepatic in the duodenohepatic ligament. This condition could have come about more easily in the course of embryonic development. 3) The small size of the left lung indicates that it never had had room to function normally and that its normal function had been disturbed early in life. 4) The small size of the inferior mesenteric artery shows an early loss of its function in supplying the colon. This creates a peculiar condition, since the blood supply of the colon in this case must come from above the diaphragm (middle colic) or from the hypogastric anastomoses in the pelvis.

Although indications are that the hernia existed at birth, there may have been some postpartum visceral rearrangement. There was no suspensory ligament of the liver present. Normally the round ligament of the liver forms the free caudal border of the suspensory ligament, and since the ligamentum teres is the remnant of the umbilical vein, it must have been present until birth. Thus its absence in this specimen must have been due to rupture or absorption following the migration or displacement of the viscera after birth.

The omentum passed through the diaphragm and between the lobes of the liver as a round cord. It passed between the lobes of the liver without any points of attachment and then onto the abdominal wall, where it formed an adhesion (*O.ad.,* fig. 5) to the right of the umbilicus. There was no change in alignment of the fibers of the transversus abdominalis muscle at the point of adhesion or any indication of scar tissue that would suggest that the adhesion had occurred during the healing of an injury.

The feeding habits of the animal must have been somewhat modified. Since the oesophagus is looped around a portion of the diaphragm, any distention of the stomach would have displaced the diaphragm and constricted the oesophagus.

The displacement of the liver to the right may have been due to the relative shifting of the attachment of the coronary ligament to the right, although it may have been accentuated by the downward movement of the left half of the diaphragm. This downward displacement of the diaphragm would have been especially great when the stomach was full. The caudal position of the right kidney is undoubtedly due to the pressure of the liver. The fact that the right ureter was not wavy suggests that it had never been of normal length and that the migration of the kidney had taken place early in the life of the individual.

The process of respiration must have been greatly modified, since only half of the diaphragm was muscular. The left half of the diaphragm could have served merely as a point of fixation when the muscles of the right half contracted during inhalation. The downward displacement could have been only on the right where some of the normal curvature remained. The downward movement of the diaphragm must have tended to pull the mediastinum to a more midsagittal position and thus to increase the right thoracic space. A sufficient movement of the mediastinum would serve to equal the increase in volume of the right thoracic space accomplished by the rib movement, and the left lung would have had little chance to function normally.

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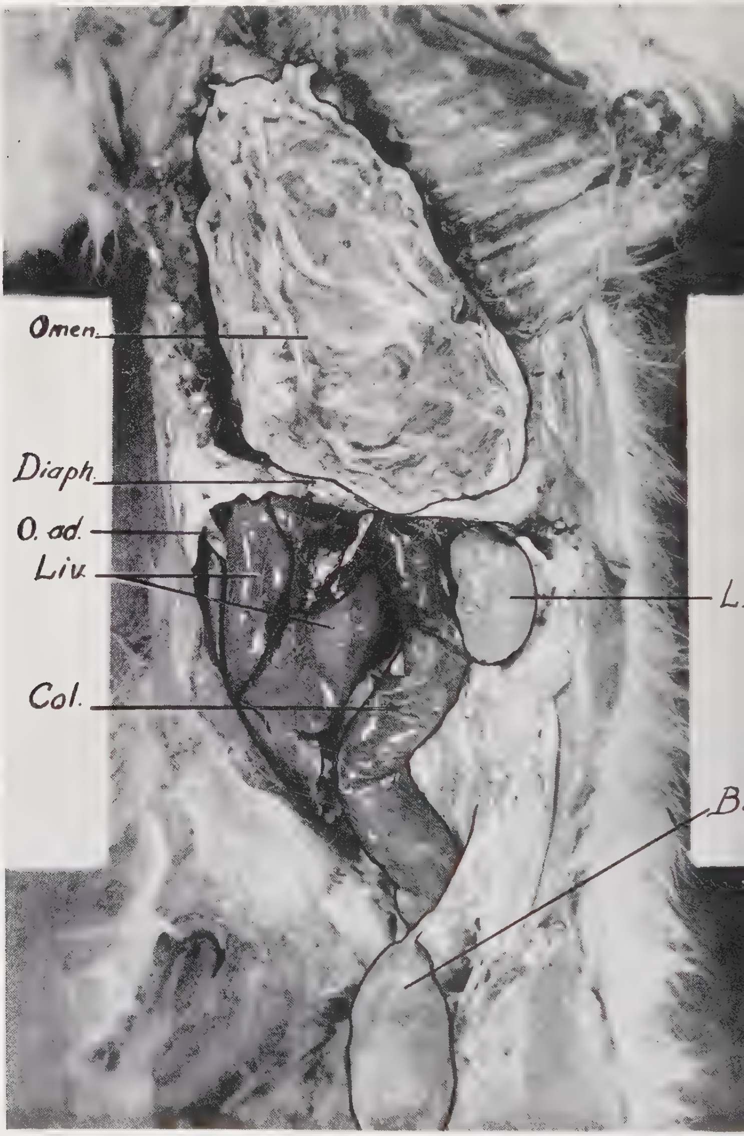
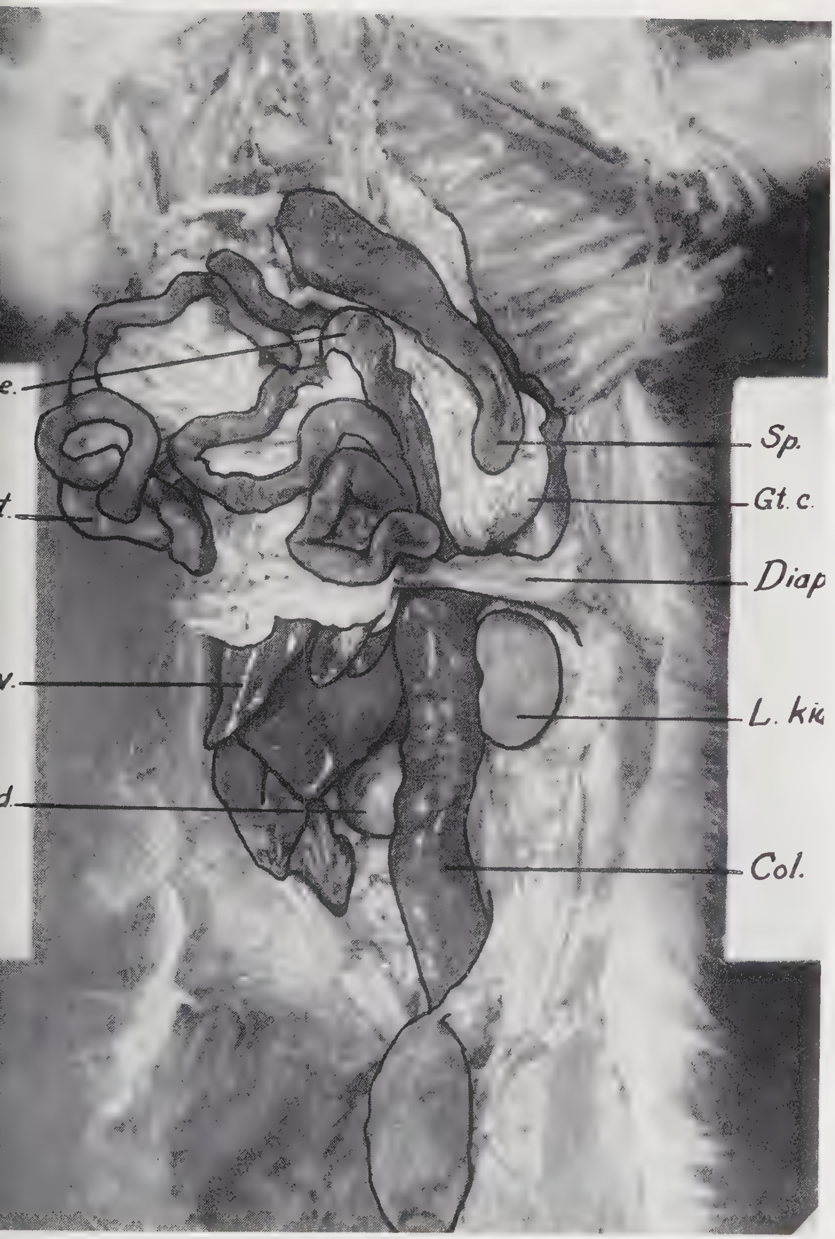


5 Photograph of the cat as it appeared when first opened. *Blad.*, b
Col., colon; *Diaph.*, diaphragm; *Liv.*, liver; *L.kid.*, left kidney; *O.ad.*,
adhesion; *Omen.*, great omentum.



Photograph showing the great omentum pulled aside, exposing the abdominal era in the thorax. *Cae.*, caecum; *Col.*, colon; *Diaph.*, diaphragm; *Gt.c.*, ter curvature of the stomach; *Int.*, small intestine; *Liv.*, liver; *L.kid.*, left ey; *R.kid.*, right kidney; *Sp.*, spleen.

ORIGIN OF THE DEEP AND CIRCUMFLEX FEMORAL GROUP OF ARTERIES

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L. R. McINTIRE, AND C. H. MARTIN
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ONE FIGURE

This paper is concerned with a discussion of origins of the deep and circumflex femoral group of arteries. The description of the arterial branches here used follows that of Senior ('24). That author based his description on embryological observations. He enumerated four vessels which constitute the deep and circumflex femoral group and defined their names as follows:

> The term a. circumflexa femoris medialis is here used in its ordinarily accepted sense, but without reference to its site of origin. The term a. profunda femoris is applied to the common stem of origin of the aa. perforantes exclusive of the trunk (of doubtful composition) by which that element of the group may be united with one or more of the circumflex elements of the group. The term a. circumflexa femoris lateralis is used here . . . . to denote the stem and ramus ascendens (B.N.A., Haller's r. transversus seu circumflexus) of the combination of two arterial elements now known as the lateral circumflex artery. As an easily recognizable substitute for the term now applied to the second element of that combination (r. descendens arteriae circumflexae lateralis, B.N.A.) the term a. circumflexa femoris descendens is here introduced.

Senior's reasons for revision of the common description of the origin of these arteries are: that in embryos of 14 to 19 mm. only one branch of the four, the a. circumflexa femoris lateralis, is definitive; that other direct branches of the newly developed femoral artery later undergo retrogression and become insignificant; and that meanwhile three secondarily enlarged routes become recognizable through the more proximal part of the rete femorale as the a. profunda femoris, the a. circumflexa descendens, and the a. circumflexa medialis.

(In the following discussion the English form of the Latin terms for the four arteries will be used: viz., medial circumflex femoral artery, deep femoral artery, lateral circumflex femoral artery, and descending circumflex femoral artery.)

MATERIAL

The cadavers which furnished the subject matter for this study were dissected in the course of routine class work in the Department of Anatomy of Washington University School of Medicine during the years 1923–24, 1924–25, 1925–26, and 1929–30. The observations were made by student dissectors and in all cases were checked on the cadavers by instructors. On the right side in white males fifty-five observations were recorded; on the left side, fifty-two. In negro males twenty-nine records were available for the right side, and thirty for the left. At our disposal there were: regardless of side, 107 arterial patterns of white males and fifty-nine of negro males; regardless of race, eighty-four rights and eighty-two lefts; regardless of race and side, a total of 166 records.

METHOD

The various types of origin of the deep and circumflex femoral group of arteries observed in the present study are shown in figure 1. The following explanatory comments may be made:

As represented in the material available for study, the four arterial branches in question may be derived from the femoral artery in the following ways: by three separate branches, as in types 1 and 1a[1]; by two separate branches, as in types 2, 3, and 5; by one common branch, as in 4 and 4a.

---

[1] In the cadavers studied, no example was seen of four separate branches of the femoral artery.

The direct or indirect derivation of the individual branches from common stems is indicated in figure 1 and needs no comment, save that in any of the cases a common stem may be short or long, but no attempt is made to differentiate between varieties which vary only according to such length of stem. For example, there is found in no small number of

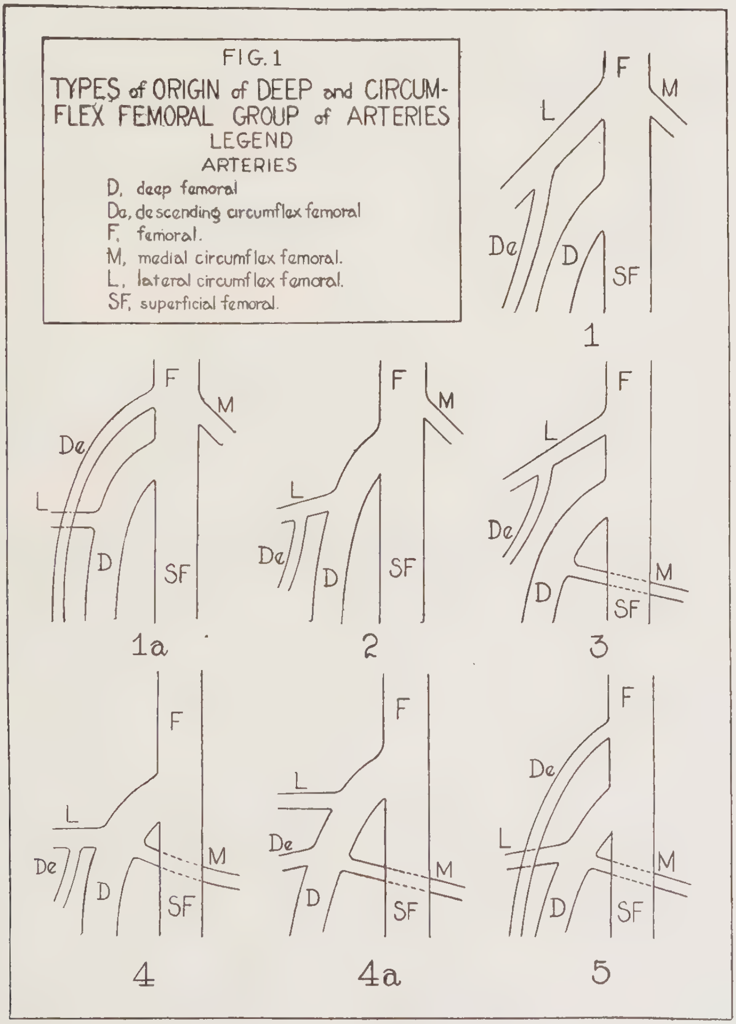

FIG. 1

TYPES of ORIGIN of DEEP and CIRCUMFLEX FEMORAL GROUP of ARTERIES

LEGEND

ARTERIES

D, deep femoral
De, descending circumflex femoral
F, femoral.
M, medial circumflex femoral.
L, lateral circumflex femoral.
SF, superficial femoral.

cases a variety of type 4 in which the medial circumflex, the lateral circumflex, and the deep femoral arteries arise from the femoral artery almost at one point by the shortest of common stems. It is assumed in this paper, upon the authority of Senior ('24), that such short stems or long ones as shown in figure 1, type 4, are very probably composite, and are not necessarily to be considered as representing the proximal part of the profunda artery.

Other types than those depicted in figure 1 undoubtedly occur, but are not found in the material available for this study. Such types would be easily provided for in the classification here presented.

DISCUSSION

Table 1 represents the result of compilation of the Washington University data on origins of the deep and circumflex femoral group of arteries according to the classification shown in figure 1.

Examination of table 1 brings forth first the question as to how well the percentages given compare with those of other authors for similar material. Adachi ('28) presents on page 152, volume 2, of his admirable work a table which sums up the results of several observers. They are: Quain ('44), Srb ('60), Ruge ('95), Auburtin ('05), and Lipshutz ('16). Comparing the percentages of white males of table 1 (average percentages of right and left sides) with those of the authors named, it is found that for each type of origin the proportions here presented fall within the range of values of the various writers. The Washington University percentage for type 4 is exceeded only by that of Srb (62.0 per cent), while Quain gives a value of 51.3 per cent. Lipshutz's material consisted of white and colored males and females, so that his values are therefore not comparable with those for the white males of this paper.

From the foregoing, it is possible to say that the material of this paper appears to be not unrepresentative of white males in general.

The number of cadavers examined in the case of white males is probably large enough to warrant certain tentative conclusions. The number of negro male bodies seen is not so great and conclusions drawn from a sample of such size are necessarily tentative. It is the hope of the authors that the investigation will continue from year to year until a large sample of the St. Louis negro population becomes available for study.

TABLE 1

Percentage incidence of the various types of origin of the deep and circumflex femoral group of arteries

TYPE	WHITE MALE		NEGRO MALE		AVERAGE PER CENT OF					JAPANESE (ADACHI)		
					R. and L.		W. and N.		R.& L., W.& N.	Male	M. and F.	
	R. (55)	L. (52)	R. (29)	L. (30)	White male	Negro male	R.	L.		R. & L., 268	R., 180	L., 187
1	9.1	1.9	6.9	3.3	5.5	5.1	8.0	2.6	5.3			
1a	0.0	0.0	3.4	3.3	0.0	3.4	1.7	1.6	1.6			
	9.1	1.9	10.3	6.6	5.5	8.5	9.7	4.2	6.9	3.7	3.9	3.2
2	27.2	17.3	10.3	20.0	22.2	15.2	18.7	18.6	18.6	16.4	16.1	13.9
3	14.5	11.5	20.6	3.3	13.0	11.9	17.5	7.4	12.4	14.6	16.1	12.8
5	0.0	3.8	0.0	0.0	1.9	0.0	0.0	1.9	1.9	2.2	3.3	1.1
4	47.3	61.5	58.6	70.0	54.4	64.3	52.9	65.7	59.3			
4a	1.8	3.8	0.0	0.0	2.8	0.0	0.9	1.9	1.4			
	49.1	65.3	58.6	70.0	57.2	64.3	53.8	67.6	60.7	61.9	58.9	67.4
							Other types,			1.2	1.7	1.6

The following tentative conclusions in regard to incidence of types of these arteries are reached upon examination of table 1:

Types 1 and 1a, the three-branched representatives, are seen more often on the right side than on the left. No real difference between the races is apparent.

Type 2, in which the medial circumflex femoral artery arises separately, occurs more frequently in white males on the right side, but in negro males, on the left. Type 3 has a greater right-sided incidence and especially is this true in negroes. Type 5 is of infrequent occurrence.

Type 4 is the variety of origin commonly described in the text-books. There is evidently a marked tendency for this form of origin to appear more often in the left thigh than in the right. Even though the incidence is definitely higher in negro males than in whites, the left-sided bias remains.

Adachi's statistical tables for right and left sides of the bodies of Japanese are based upon a population of about three-quarters males and one-quarter females. Our findings agree with his: in that type 4 (Adachi's *Truncus profundo-circumflexus perfectus*[2]) is oftener seen on the left than on right; in that type 3 (Adachi's *Truncus profundo-circumflexus medialis*) is more frequently a right-sided than a left-sided phenomenon; there may be a difference as to sidedness in the occurrence of type 2 (Adachi's *Truncus profundo-circumflexus lateralis*). (Type 2 appears to be found oftener on the right side in Japanese and in whites, but oftener on the left in negroes.)

The authors do not mean these statements as to differences in incidence according to race or to side of body to be accepted as final; the number of observations involved does not warrant that. However, it is clear that there is great probability of the existence of such differences. If that is true, percentage tables relating to incidence of the various types of origin of these arteries should show segregation of the data according to side of body and according to race. If it is desired to present percentages regardless of side or race, equal numbers of each side or of each race should be used in the calculations; or average percentages of the two sides should be calculated for the races, and average percentages of the races for the sides of the body. If, for example, one desires to calculate from the data of table 1 the incidence of type 2 on the right side of the body regardless of race, it is incorrect to add the number of cases of whites of type 2 to those of the negroes of type 2 and to divide that sum by the total number (84) of white and negro patterns (55 and 29, respectively) of the right side. Such procedure is incorrect, because there are twice as many whites as negroes, and the resulting proportion is biased by the larger number of whites. The simplest correct method is to average the percentages for white males and negro males for the right side. In this manner the difficulty of difference in size of sample is avoided.

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[2] "I think the term 'perfectus,' originally used by Barkow, is objectionable in the same sense as is the description of the 'normal' profunda and its branches given in text-books. It distinguishes between the 'perfect' or 'normal' form and 'imperfect' or 'abnormal' forms. It, in fact, tends to preserve a rigidity in description where plasticity is required.

"If a *truncus profundo-circumflexus* is the *only* large muscular branch of the femoral it is necessarily a *truncus profundo-circumflexus medialis et lateralis et descendens* (or a *truncus profundo-circumflexus perfectus*), and the unqualified term *truncus profundo-circumflexus* is sufficiently clear. Even when there are one or two separate direct muscular branches, the mere naming of the smaller branch or branches indicates the nature of the common trunk. This is, however, referred to as a *truncus profundo-circumflexus imperfectus* or *truncus circumflexus communis,* according to circumstances. In the case of two common trunks, one must necessarily be a *truncus profundo-circumflexus* and the other a *truncus circumflexus communis,* in which case the constituent branches of the latter would require to be named" (Senior, H. D., '30; personal communication).

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CHONDRIOME AND VACUOME OF THE DIFFERENTIATING ERYTHROCYTE OF NECTURUS AND THEIR RELATION TO THE SO-CALLED GOLGI SUBSTANCE OF ERYTHROCYTES

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ONE PLATE (NINE FIGURES)

In an earlier paper a brief and incomplete description of the chondriosomes of the erythrocytes of Necturus was given (Dawson, '28). At that time the features of main interest were the morphology and staining reactions of the so-called segregation apparatus, which now appears to be homologous with the vacuome described by Parat and fellow workers as typical of many animal cells. However, in the erythrocytes of Necturus the bodies reacting with neutral red appear to be of greater density than the surrounding cytoplasm and might more appropriately be called granules rather than vacuoles. Only scant attention was given to the chondriosomes. They were then described as granular, but frequently grouped in short chains, and no characteristic distribution of these elements was noted. Subsequent observations indicated that this brief description was so incomplete as to be almost inaccurate, but it was only recently that the material for a satisfactory study of the chondriosomes was obtained.

As shown in a previous study (Dawson, '30 b), the erythrocytes of Necturus undergo extensive degenerative changes following the immersion of these animals for several days in a solution of lead acetate (1: 20,000). The injured erythrocytes are rapidly removed by phagocytosis which is so intense that great numbers of macrophages are even encountered in the circulating blood. Following the anemia produced by the lead poisoning, there is a period of active regeneration of red blood cells and practically all stages of the differentiating erythrocytes are found in the circulation. Abundant mitoses of these elements were also observed, the blood picture resembling closely that described for several urodeles in the physiological regeneration which apparently occurs in early summer (Dawson, '30 a). In such blood it was therefore possible, by means of supravital staining with neutral red and Janus green B, to study without difficulty the morphology and distribution of both the chondriosomes and the neutral-red granules.

Much of the blood on which this study is based was accordingly obtained from animals recovering from the effects of lead poisoning. This is especially true of the several stages in the differentiation of the erythrocyte. Studies of the mature erythrocyte were also made from blood of normal animals, and in the case of the mature erythrocyte the observations obtained by means of the supravital technic were supplemented by fixed material prepared by the method of Champy-Kull. In many instances it was possible to first study a cell by supravital methods and later, after having dried and stained the preparation, to view the same cell stained with a differential blood stain (Wright's or Giemsa's). Characteristic cell groupings and care in leaving the mechanical stage undisturbed aid greatly in making such identifications.

In this description reference to the parent cell of the erythrocyte is purposely omitted, as the study of this phase of the problem is still incomplete. Accordingly, only the observations made on cells which have begun their differentiation will be included. Such cells are readily recognized in fresh preparations by several characters, such as form, nuclear-cytoplasmic proportions, texture of cytoplasm, and the presence of perceptible amounts of hemoglobin. Hemoglobin in minute amounts may be recognized in fresh cells even when it is obscured in fixed and differentially stained smears by the marked basophilic reaction of the cytoplasm.

DESCRIPTION

The earliest stage of erythrocytic differentiation readily recognized in supravitally stained preparations is illustrated in figure 7. Such cells were relatively small and contained only the faintest trace of hemoglobin. The cytoplasm appeared clear and contained but few mitochondria, which were in the form of short, blunt rods and were usually grouped in the vicinity of the nucleus. Neutral-red bodies were few, very minute, and scattered.

Cells of this stage of differentiation were not numerous in any of the preparations examined and apparently they only make their appearance early in the regenerative process. They represent a stage of differentiation somewhat younger than that described and illustrated (figs. 5 and 6) in the study by the author (Dawson, '30 a) on the physiological regeneration of the blood of urodeles. The nuclear pattern resembled that of the lymphocyte, but the cytoplasm, while strongly basophilic, possessed a turbidity which is not found in typical lymphocytes. Cells of this degree of differentiation were not observed in mitosis, while cells only slightly more differentiated were frequently seen undergoing indirect division.

Figure 6 illustrates the next stage in the development of the red blood cell. The chondriosomes of these cells are still relatively large, but most have assumed a spherical form. They are relatively numerous and are grouped about the nucleus, showing a slight tendency to accumulate at its poles. The neutral-red bodies are few, small, and irregularly distributed. Besides these bodies which react with either Janus green or neutral red, clusters of larger refractive bodies are occasionally also seen. In differentially stained smears these bodies appear as vacuoles. Their significance is not known. The cytoplasm of these cells is intensely basophilic and has a coarse granular appearance.

Other cells are present which appear slightly more advanced in development (figs. 4 and 5). They are either circular or oval in outline and have an irregular outline, and their surface is seen to be marked by rounded protuberances. The mitochondria and neutral-red bodies resemble those of the preceding stage in almost every respect. In stained smears the nuclear pattern resembles that of the mature erythrocyte except that the meshes of the reticulum are wider and more distinct. The cytoplasm is still decidedly basophilic, appears flaky, and is marked by creases which are due to the surface irregularities so clearly seen in fresh preparations. These cells are frequently seen in mitosis.

In the later stages of regeneration the most numerous type of cell encountered is a large, oval cell with considerable hemoglobin within its cytoplasm (fig. 3), although in stained smears this still tends to be obscured by the massive basophilic reaction. The chondriosomes are large, numerous, and spherical in form, and exhibit a uniform tendency to polar aggregations, while the neutral-red bodies are relatively few, small, and diffusely scattered through the cytoplasm. The nuclear pattern resembles closely that of a mature cell, but the cytoplasm in stained preparations is still flaky in appearance. These cells also divide by mitosis.

The other atypical erythrocyte found in the regenerating blood is very large, being relatively long and narrow with blunt ends and nearly parallel sides (fig. 2). The amount of hemoglobin in the cytoplasm (as seen in fresh preparations) is not increased over that of the preceding stage, but in differentially stained preparations the basophilia is found to be greatly reduced and the flakiness of the cytoplasm has been replaced by either a fine granular or homogeneous appearance. The spherical chondriosomes are strikingly increased in number, reduced in size, and frequently occupy almost completely the cytoplasm accumulated at the two poles of the cell. The neutral-red bodies, however, are not appreciably increased in number or size and are also diffusely scattered. These large cells are occasionally encountered undergoing mitosis.

There are many intermediate stages between these large cells and the typical mature erythrocytes (fig. 1). The reduction in size is apparently accomplished by mitosis and the concentration of hemoglobin is also gradually increased. The number of chondriosomes is, however, gradually reduced and they frequently become grouped into small clusters. They later lose their tendency to bipolar distribution and come to occupy a narrow zone of cytoplasm immediately encircling the nucleus. The neutral-red bodies also lose their diffuse distribution and become restricted to two definite clusters located near the two poles of the nucleus. At this time the clusters of chondriosomes assume the form of filaments, which when critically examined are often found to be beaded, indicating their mode of development from discrete, spherical elements. In fresh preparations these filamentous forms exhibit slow movements, continuously twisting, turning, and bending.

The changes discussed above do not always occur in such a definite sequence. Cells are frequently observed with a relatively high concentration of hemoglobin and with definite clusters of neutral-red granules, but the chondriosomes are still present as discrete spheres, either scattered or grouped. At other times the mitochondria may be filamentous and definitely grouped about the nucleus, but the neutral-red bodies may not be definitely clustered in the regions of the poles of the nucleus. Occasionally both the mitochondria and neutral-red bodies have the form and distribution characteristic of the mature cell, but the hemoglobin may not have reached the maximum concentration.

The decrease in the number of mitochondria in the cell is apparently associated with attainment of the maturity of the cell. The restricted distribution of both the mitochondria and neutral-red bodies appears to be correlated with the assumption by the cell of its typical form and the attendant increase in the viscosity of the cell contents. The typical form of the mature cells is familiar to all. They are disc-shaped bodies, with a central convexity on both sides, due to the presence of the nucleus, and peripheral to the convexity there is a broad flat flange. Accordingly, the cytoplasm of the erythrocyte is in all parts of the cell a relatively thin layer, with a maximum thickness about the periphery of the nucleus as the cell is viewed en face, growing gradually thinner toward the edge of the cell, and probably being thinnest between the surface of the nucleus and the cell wall in the regions of the two convexities. In other words, both the mitochondria and neutral-red bodies occupy the region of the cell in which the cytoplasm has its maximum thickness.

From his studies, by means of microdissection methods, Seifriz ('26) concluded that the form of the amphibian erythrocyte is due to, and maintained by, its semirigid wall. The region of the so-called flange constitutes the greater portion of the cell wall and in this zone the cytoplasm probably reaches its minimum thickness and greatest viscosity. The physical condition of the cytoplasm in the region of the flange would therefore tend to maintain the restricted distribution noted for both the mitochondria and neutral-red bodies, even when the cell is deformed in its passage through the small blood vessels.

Further evidence that the central cytoplasm immediately about the nucleus differs from the more peripheral cytoplasm is to be found in the observations of Hartman ('27) and Jordan, Kindred, and Beams ('30). In both instances the method of Golgi (gold-chloride impregnation) for the demonstration of the special body within the erythroplastids of mammals was employed. According to Hartman, in avian and reptilian corpuscles the gold-chloride-staining material surrounds the nucleus and appears less granular than the central body of the mammalian cells, the gold apparently being deposited on the basis of the hemoglobin in the corpuscles which have been fixed with strong potassium bichromate and mercuric chloride. Jordan, Kindred, and Beams have demonstrated a similar zone in the red cells of amphibians and have referred to the structure as the reticulofibrillar apparatus of Golgi and suggest it may represent a denser or more robust perinuclear stroma.

In studying fresh blood it is difficult to convince oneself that there is relatively more hemoglobin in the cytoplasm of the perinuclear zone. There is probably absolutely more hemoglobin in this region, since the volume of the cytoplasm is greatest about the nucleus and, as Hartman has pointed out, there is no evidence that the hemoglobin has not been materially altered either in character or position by the fixation and treatment in the special method of Golgi. The idea of a supporting stroma in erythrocytes is rapidly losing ground and these cells are now rather generally regarded as sacs filled with a fluid or semifluid substance. The observable differences in the physical state of the perinuclear and peripheral cytoplasm are probably sufficient to account for the rather precise way the gold chloride is precipitated within the cell and its apparently sharp delimitation of a perinuclear structure.

As has been previously pointed out (Dawson, '28, '29), exposure of normal erythrocytes to high concentrations of neutral red, especially under unnatural conditions, results in the neoformation of many bodies which also stain with this dye. In most mature cells these bodies increase in number and coalesce to produce elongated filaments which in turn frequently anastomose to produce the pattern of reticulation.

In the younger stages of erythrocytopoiesis the same phenomenon of induction of neutral-red bodies is observed. These, as they increase in size and number, do not coalesce to produce filamentous structures, but remain as discrete globules (figs. 8 and 9). There therefore appears to be quite a definite correlation between the presence of spherical chondriosomes in the cytoplasm and the tendency of the induced neutral-red bodies to fail to coalesce and produce filaments or patterns of reticulation. That is, the physicochemical state of the cytoplasm which favors the agglutination of spherical chondriosomes into chains or beaded filaments appears to permit a like change in the induced neutral-red bodies. This tendency for aggregation of disperse bodies is also seen in the case of the normal preexisting granules which are widely dispersed during the differentiation of the erythrocyte, but become grouped as two bipolar clusters in the mature cell.

DISCUSSION

The incomplete account given in an earlier paper (Dawson, '28) of the mitochondrial distribution in the erythrocyte was largely due to the difficulty of distinguishing these elements, even when supravitally stained, on account of the thickness of the cell and the presence of the hemoglobin. However, by using a ribbon-filament mazda lamp in conjunction with a Corning 'Daylite' glass-and-water filter, a sufficiently intense illumination was secured to enable the mitochondria to be readily observed even with a binocular microscope. In the earlier study, only in cells with a low hemoglobin content could the mitochondria be clearly observed, and consequently chiefly the granular forms were observed, although short chains were recognized. The filamentous form, often appearing beaded, has also been successfully demonstrated by the Champy-Altmann-Kull method in the mature blood cells. In such preparations the cytoplasm of the erythrocyte appears hyaline and glassy and is stained yellow by the aurantia, while the mitochondria are colored a deep red by the acid fuchsin. Filamentous mitochondria have also been reported for the erythrocyte of Necturus by Shipley ('15).

In a recent study of the cytology of the erythrocytes of the giant salamander, Kurashige ('30) describes the Golgi substance as usually appearing in the form of threads, but sometimes even in granular form. The Golgi substance, according to this observer, turns into special granular substance, which afterward liquefies and becomes vacuolar and is then expelled from the cell body. The Golgi substance is believed by Kurashige to be derived from a transformation of nucleolar substance which transudes through the nuclear membrane and appears first as specks. He regards all this as evidence of metabolic activity and states that changes from speck to filament, granule, and vacuole are continuous phases and that the phenomenon is repeated in each cell at irregular intervals.

If my observations on the 'segregation apparatus' (Dawson, '28) were added to the observations recorded in this paper, it would seem that the same types of formed elements occur in the erythrocytes of Necturus as in the giant salamander, but the interpretation of these structures by Kurashige differs widely from my views on this subject.

Kurashige states that he is unable to demonstrate any mitochondria in the erythrocytes of the giant salamander even with the method of Champy-Altmann-Kull, and he refers also to similar difficulties encountered by Avel ('24) in dealing with the blood of other amphibians. In this respect it seems significant that both Avel and Kurashige have described a filamentous form of Golgi substance. In my early discussion of the subject I felt that the so-called filaments of Golgi substance might be the result of the impregnation of an early stage of reticulation, but it now appears that there is a further likelihood of filamentous mitochondria being impregnated by silver and osmic methods, thereby simulating the Golgi apparatus. Furthermore, in the Sauropsida the normal neutral-red bodies have been impregnated by both silver and osmic methods and have been described as Golgi bodies (Bhattacharya and Brambell, '25).

After several years of rather intensive study of the erythrocyte of Necturus in which a wide variety of technical methods have been employed, I have been unable to discover any special, formed element other than those readily disclosed by the accepted technical methods. Furthermore, this erythrocyte of the blood stream cannot be properly represented as a senile cell, lacking certain special structures. It possesses a nucleus, mitochondria, and neutral-red bodies and retains the ability to divide by mitosis up to a relatively late stage of differentiation. Cells have been observed in mitosis which differed from fully mature cells only in the persistence of a slight basophilia in the cytoplasm.

It was hoped that in such a study as this some light would be thrown on the mechanism of the elaboration of hemoglobin. The neutral-red bodies, however, outside of the changes in their distribution throughout the cytoplasm, exhibit little change during the period of synthesis of hemoglobin. The chondriosomes, on the other hand, show definite, and sometimes very striking, changes in size, form, number, and distribution.

Very similar changes in both these elements have been described by Sabin and collaborators ('25 a, b, and c) for the several cells of the blood of birds and mammals. Sabin feels that changes in the form of the chondriosomes are probably not so significant as changes in distribution, size, and number. Diffuse arrangement with wide distribution through the cytoplasm and small size characterize the mitochondria in the growth phase preparatory to cellular division. In earlier stages of the growth phase the mitochondria may be larger and clumped, usually about the nucleus. In general, Sabin finds that the presence of mitochondria in relatively large numbers may be the sign of a young or a mature blood cell, and that their absence may be correlated either with a very undifferentiated stage or with a senile stage in which there is little or no capacity to divide. In the birds and mammals the neutral-red bodies are gradually reduced in number, being represented by a few granules in the mature avian erythrocyte and completely lacking in the definitive mammalian erythroplastid.

Apparently in the differentiation of the several cellular elements of the blood the chondriosomes undergo much the same general changes in size, number, and distribution, and these changes are in each case associated with the specific differentiation of each type of blood cell; so that there does not seem to be any special significance in the history of the neutral-red bodies and chondriosomes as outlined for the erythrocyte of Necturus. It is true that the differentiation in the erythrocyte is marked chiefly by the synthesis of hemoglobin, but there does not seem to be any specific morphological expression of the relation of either the mitochondria or the neutral-red bodies to this process. It is, moreover, a conspicuous fact that many of the cytoplasmic differentiations leading to the production of a specific end product are molecular or chemical in character rather than morphological and the only evidences obtained cytologically are the various accumulations of end products (granules of secretion, etc.) within the cell. The extent to which any of the special formed elements of the cytoplasm are involved in any intracellular synthesis is still a matter of wide diversity of opinion.

SUMMARY

Practically all stages of the maturation of the erythrocyte are encountered in the peripheral blood of Necturus during the intense regeneration which occurs following the secondary anemia produced by lead poisoning. Supravital staining methods were employed.

The youngest stages, containing but a trace of hemoglobin, possess relatively large rod-shaped mitochondria scattered through the cytoplasm. The neutral-red bodies are few, minute, and uniformly distributed.

In cells possessing still more hemoglobin, the mitochondria are always greatly increased in number and are either in the form of large or small granules. The larger mitochondria are frequently loosely grouped about the nucleus, while the smaller, granular forms are more often diffusely scattered. The neutral-red bodies are still minute, slightly increased in number, and uniformly distributed through the cytoplasm.

In the mature cell, however, the mitochondria are strikingly reduced in number and occupy a definite perinuclear position. They are in the form of either relatively long chains or filaments. The neutral-red bodies are not increased in number, but are larger and definitely grouped as two bipolar clusters.

Many of the younger cells exhibit active mitosis, and it appears that the large mitochondria (blunt rods and spheres) may be associated with the earlier growth phases, while the smaller granular forms indicate a phase of cellular proliferation.

The restricted distribution in the mature erythrocyte of both mitochondria and neutral-red bodies is probably associated with the assumption of the typical form and the accompanying increase in the viscosity of the cell contents. In the younger cells the preformed and induced neutral-red bodies do not coalesce to produce filaments or reticulations as in mature cells, but remain as discrete globules.

It is suggested that the filamentous form of the Golgi substance described by Kurashige ('30) for the erythrocytes of the giant salamander may be due to the impregnation of filamentous chondriosomes, since this author was unable to demonstrate mitochondria in the erythrocytes studied by him.

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EXPLANATION OF PLATE

All figures were drawn at the same magnification and were outlined by means of a camera lucida. Mitochondria, gray; neutral-red bodies, black; unstained vacuoles or globules, circles. Neutral red with Janus green B, applied supravitally, used in all cases.

PLATE 1

EXPLANATION OF FIGURES

1 A mature erythrocyte, showing the form, size, and distribution of the neutral-red bodies and mitochondria.

2 A large atypical erythrocyte containing numerous small spherical chondriosomes and scattered neutral-red bodies. Concentration of hemoglobin decidedly below normal.

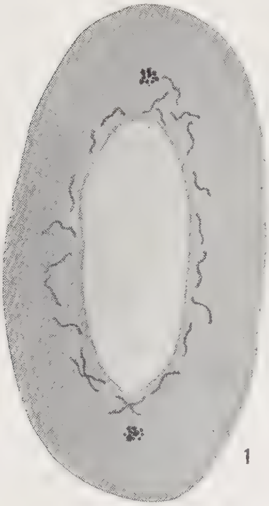
3 Immature erythrocyte, showing characteristic distribution of large spherical chondriosomes and neutral-red bodies.

4 and 5 Immature erythrocytes, showing the irregular appearance of the surface of the cell. The chondriosomes are grouped in the perinuclear region. The neutral-red bodies are irregularly scattered.

6 A slightly younger stage than figure 5. This cell contains a few unstained globules or vacuoles besides the characteristic neutral-red bodies and chondriosomes.

7 A small immature form with only a small trace of hemoglobin. The chondriosomes are few in number and usually have the form of short blunt rods. Neutral-red bodies few and scattered.

8 and 9 Two immature cells showing the effect of prolonged exposure to high concentrations of neutral red. The neutral-red bodies are increased both in size and in number, but tend to remain separate, in contrast with the tendency to fuse which is exhibited by similar induced bodies in the mature red cell.



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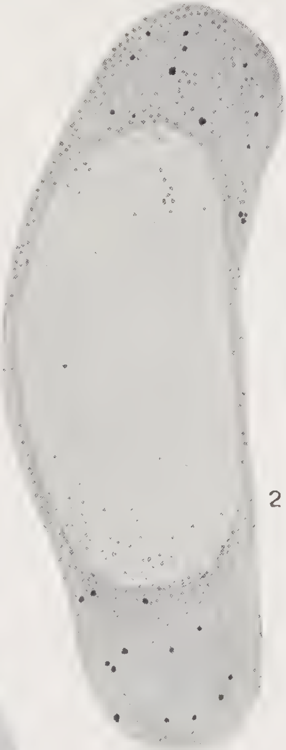

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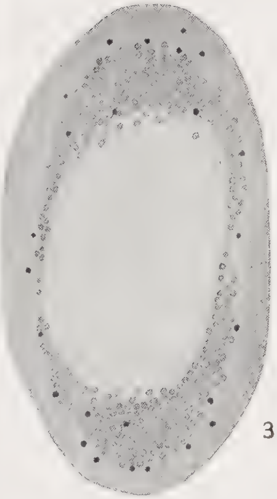

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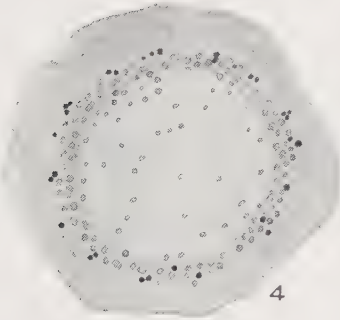

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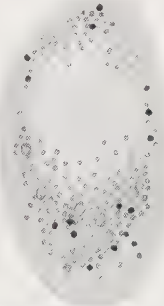

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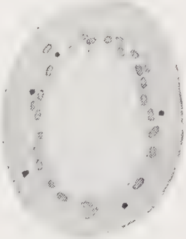

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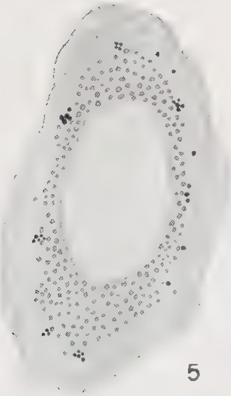

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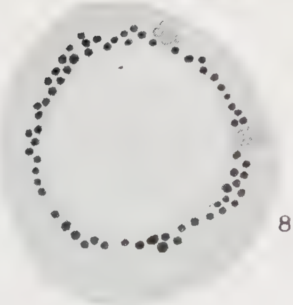

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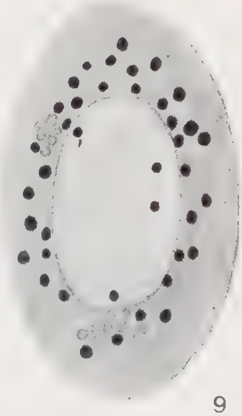

9

AN INVESTIGATION OF THE STAINING REACTIONS OF ERYTHROCYTES OF THE LEOPARD FROG TO NILE-BLUE SULPHATE, WITH SPECIAL REFERENCE TO THE NATURE OF THE SEGREGATION APPARATUS AND THE GOLGI SUBSTANCE

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THREE FIGURES

The methods employed were based primarily on the work of Scott ('28) on the vital staining of blood cells. By staining frog erythrocytes with Janus green and neutral red, in accordance with his method, results were obtained similar to those described in his article. The slides showed immediate staining of the neutral red granules and mitochondria, and the former gradually increased in number. With the concentration used (1 per cent), there was no evidence that the neutral red granules either ran together or faded on long standing or in permanent preparations.

The question of the identity of these neutral red granules (the so-called 'segregation apparatus') with the Golgi apparatus has been discussed recently by several workers. Dawson ('28), from the results of previous work by others and from his own experiments on the blood of Necturus, concludes tentatively that "the substance of the 'segregation apparatus' is of the same nature as the Golgi substance." The emphasis with Dawson was placed upon this identity. An acceptance of this theory, against which no significant evidence has been produced, makes it possible to apply conclusions reached concerning the recently defined segregation apparatus equally to the historically recognized Golgi substance.

A further step would be an investigation of the chemical nature of the granules. It has been suggested that the Golgi substance is lipoidal. Dawson used as vital stains neutral red and brilliant cresyl blue. While these dyes are known to stain fats, they are not specific for them. Nile-blue sulphate (diethyldiaminophenazoxonium sulphate) was suggested by the work of Tennent ('28) on material extracted from the ovaries of Echinometra lucunter. J. Lorraine Smith ('07) had shown that this dye stained neutral fat red, and fatty acid blue. Tennent found that the presence of even a small amount of fatty acid mixed with neutral fat will cause a blue color. "The blue staining therefore can not be regarded as necessarily indicating an absence of neutral fat." Nevertheless, this dye is specific for these substances in so far as they may be considered as two forms of one basic substance.

The form of the segregation apparatus is customarily described as 'granules and globules,' and has been proved by Jordan ('28) to exist in unstained erythrocytes. A complex 'segregation apparatus,' consisting mainly of a network, has also been said to preexist and to be formed by the coalescence of these granules. This point has been considered fully during the present experiments by continuous observation under the microscope of definite groups of corpuscles. The authors feel convinced that the reticular form of the apparatus is the result of fusion of the granular material due to degenerative processes in the cells.

The experiments were suggested by Prof. David H. Tennent and were carried out under his supervision.

DESCRIPTION OF THE EXPERIMENTS

The blood was taken from the heart of unetherized frogs, in order to get blood free from any skin secretion that might hasten death changes. Solutions in absolute alcohol of Nile-blue sulphate were made in varying concentrations, the significance of which will appear later. A drop of the solution was placed on the end of one slide and spread across it with another. The rapid evaporation of the alcohol left a thin layer of the dry dye. By using equal pressure and speed, it was possible to get uniformly covered slides showing distinct color gradations from a pale to a dark blue. In making the preparations the heart was punctured and a cover-slip pressed against the frog's heart, so that the whole cover-slip was covered with a thin film of blood. This was then placed in the center of a prepared slide and pressed lightly, until there was a firm adherence to the slide. The edges were then sealed with vaselin. Preparations such as these would remain moist enough for the erythrocytes to flow even after twenty-four hours. The results appeared to vary directly with the concentration of the dye. This in turn was determined by several factors, namely: the strength of the solution used, the amount of blood, the degree of pressure applied to the cover-slip before sealing, and the evenness of distribution of the blood on the slide. Although all possible care was taken to make the slides uniform, nevertheless differences in degree of staining were always found even within the limits of one slide. However, this variation was not entirely disadvantageous, since it permitted of more easy comparison between lightly and heavily stained cells. The results as here stated for each concentration will therefore represent as nearly as possible the most typical effects.

0.5 per cent to 1 per cent

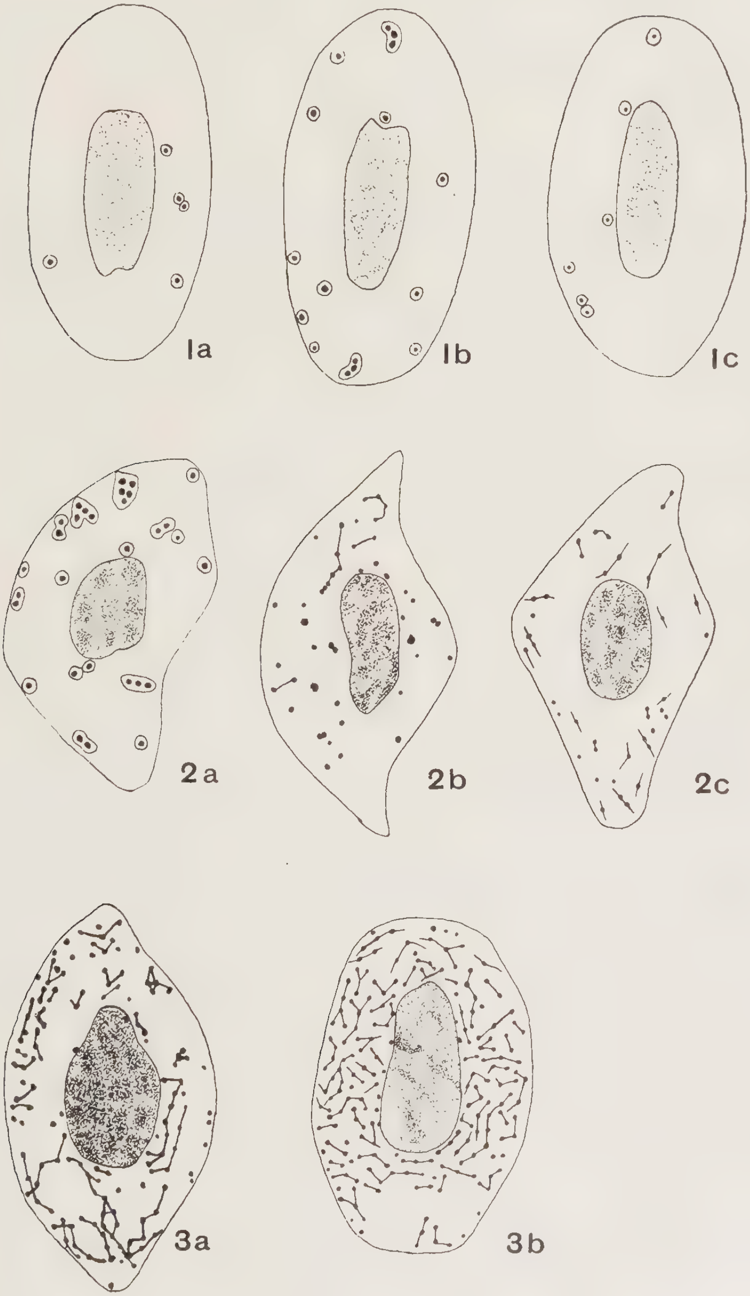
A few granules were immediately stained blue (fig. 1, a) and surrounded by clear 'halos' or vacuoles. The nucleus showed practically no coloration and the cytoplasm was a pale greenish-blue. During the first twenty minutes the granules increased in number until there were about fifteen or twenty in each cell. They showed an oscillatory motion in the apparently fluid cytoplasm, often moving quite rapidly along the periphery. Even in this weak concentration there was a tendency for the granules to arrange themselves in rows of three or four, now moving together, now separating for a short distance to return again (fig. 1, b). At the end of three hours the oscillation had ceased and there was a marked disappearance and fading of the granules, so that, in most cases, only five or six, small and rather faintly colored, remained (fig. 1, c). In these cases there was no perceptible distortion of the shape of the cells.

1.5 per cent to 2 per cent

The same process of accumulation of granules with time was noted. Here, in many cells were found larger aggregates, —groups of five or six granules which persisted in staying together in spite of oscillatory movement (fig. 2, a). It was here, too, that there appeared for the first time a phenomenon which became of great significance later, namely, the appearance in some cells of fine thread-like interconnections between granules (fig. 2, b and c). It is difficult to picture these strands accurately by drawings, as they lie in many planes and at many depths in the cell. In the more deeply stained corpuscles in these preparations there was considerable shrinkage of the cytoplasm and consequent distortion of shape. After standing nine to twelve hours, the nuclei rounded out until they were quite spherical and the cytoplasm resumed a more normal outline. These preparations did not show any signs of fading.

3 per cent to 4 per cent

The use of stronger solutions of dye produced more striking, although more varied, results. In some portions of the slides the cytoplasm would be destroyed, leaving only fringes around deeply stained nuclei. Some cells showed at first only a few large granules which increased in number and within twenty minutes were interconnected by threads. Others had this threaded appearance from the first (fig. 3, a and b). With this concentration, there was formed, in many cases, a heavy, unmistakable network. In no case, however, did the action proceed to such an extent that the granules from which the network arose were not still visible. The staining of the nuclei was immediate and deep, indicating a ready permeability to the dye. The effects of distortion, first by


1a 1b 1c
2a 2b 2c
3a 3b

Figures 1 to 3.

shrinkage and afterward by swelling of the nucleus and cytoplasm, were even more pronounced here than in the previous preparations.

DISCUSSION

The results of the experiments bring out several points clearly. The process of staining with Nile-blue sulphate may be described as follows: the cells show immediate staining of the preexisting granules and globules, even in slight concentrations. By taking this stain they are identified as containing fatty acid, and their deep color would indicate a high percentage of it. The granules increase in number up to a certain maximum—a phenomenon explained by Dawson ('28) as due to gradual formation of new granules which are then stained. The behavior after this depends upon the concentration of the dye, and this fact is significant in clearing up the questions concerning the complexity of a preformed segregation apparatus.

It was first noted that a 3 per cent to 4 per cent solution of the dye, definitely toxic to the cells as shown by distortion or complete cytolysis, gave slight networks immediately, or striking ones within several hours. When considered with the fact that the granules never disappeared, but persisted as definite nodes in the network, it seemed logical to suppose that the production of a reticular form of apparatus was correlated with degenerative physical or chemical changes in the fatty-acid granules. This was further substantiated by noting that no cell contained a reticulum which did not show some degree of distortion. The most heavily stained cells showed networks at once, while those lightly stained never showed any. With medium concentrations the process of formation occurred at a slow enough rate to permit observation of successive changes. Cells stained with 1.5 per cent to 2 per cent dye solution were watched at intervals during thirteen hours. Within this time the 'apparatus' of each passed through stages from separate granules to aggregates and then to sparsely interconnected granules. These interconnections were in many cases elaborated into a network. Thus continuous observation of one cell left no room for doubt that the reticulum came directly from the granules.

The so-called 'segregation apparatus' of frog erythrocytes is therefore reduced to granules and globules, and to all appearances any other form is an artifact. If the Golgi apparatus is completely identifiable with the segregation apparatus, it becomes merely a collection of fat-containing inclusions, and not a cell organ. This suggests that it may be an aggregation of products of cell metabolism, transitory, although always present in some degree.

SUMMARY

1. The granules of the segregation apparatus can be vitally stained with Nile-blue sulphate as well as with neutral red and brilliant cresyl blue.

2. Nile-blue sulphate has the advantage of being, in a sense, specific for neutral fat and fatty acid. The reaction is blue for fatty acid, however slight the amount, and all the experiments gave blue granules.

3. Networks are produced from preformed granules in the cells, either by their rapid destruction from too strong a concentration of dye, or gradually, due to death changes following isolation from the body.

4. The segregation apparatus thus becomes reduced to granules and globules of fatty-acid composition, wholly or in part, which immediately suggests one of the transitory phases of a metabolic process.

5. If the segregation and Golgi apparatuses are identical, as has been strongly suggested, the foregoing conclusions can be applied to the Golgi substance.

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GOLGI APPARATUS, CANALICULAR APPARATUS, VACUOME, AND MITOCHONDRIA IN THE ISLETS OF LANGERHANS OF THE ALBINO RAT

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TWO PLATES (SIX FIGURES)

INTRODUCTION

A little over three decades ago, Commila Golgi ('98) first described the 'apparato reticulare interno' in the cytoplasm of the Purkinje cells of the cerebellar cortex of the owl's brain. A few months later, he found the same apparatus in the spinal-ganglion cells of a number of mammals. Golgi's students and others immediately extended his silver methods to many kinds of cells and were successful in demonstrating the apparatus which now bears his name. Golgi was always very cautious in expressing his view on the nature of his new discovery. However, he was of the opinion that the 'internal reticular apparatus' consists of a close network of fine fibrils, located in a fairly definite region of the cytoplasm intermediate between the nucleus and the cell surface. He interpreted the net as entirely intracellular, having no direct communication with the extracellular structures.

Since the time of the discovery of the Golgi apparatus it has been described in practically every type of cell wherever investigated (for certain exceptions, vide Cowdry, '24). However, notwithstanding extensive investigations, the nature and function of the Golgi apparatus remain largely in doubt.

Only a year after Golgi's original communication to the medical society of Pavia, Holmgren ('99) found a system of structures in the cytoplasm of nerve cells which he thought

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were identical with the new discovery of Golgi. In numerous contributions over a period of some fifteen years, Holmgren extended his studies to many kinds of tissues, and developed his theory that the system of intracellular canals arises from ingrowths of cytoplasmic processes ('trophospongium') which may become vacuolized, forming canals ('Saftkanälchen') that communicate directly with the exterior of the cell. Holmgren originally thought that the function of the 'trophospongium' was nutritive and that in the ganglion cells it ('Saftkanälchen') was connected with the lymphatic channels.

Much more recently, Parat and Painlévé ('24, a, b, c, '25), Parat ('28), and others have advanced the theory that the apparatus of Golgi and the trophospongium of Holmgren are artifacts produced by the methods employed in their demonstration. They think that the reality is a system of vacuoles and granules (vacuome) in the cells that are specifically colored by neutral red.

> Il est néanmoins certain que la 'trophosponge' résulte d'une déformation considérable de l'appareil vacuolaire et qu'elle est bien plus un artefact que l'image de la réalité ('24 b).
>
> L''appareil' cellulaire que l'on a coutume d'appeler 'appareil reticulaire' interne de Golgi ou 'trophosponge' de Holmgren résulte d'un précipité d'argent ou d'osmium métallique à l'intérieur, à la périphérie ou dans les intervalles des vacuoles protoplasmiques ('24 c).

Regarding a reticular type of apparatus they say, "lorsqu'un réseau a été decrit, il s'agit d'une confluence artificielle de vacuoles due à l'action du fixateur et renforcée par le dépôt d'argent ou d'osmium" ('25).

Still another group of investigators has found a close morphological relationship between the Golgi apparatus and mitochondria. In fact, Deineka ('14) was unable to distinguish between the Golgi material and mitochondria in bone cells. Monti ('15) concludes that the Golgi apparatus and the chondriosomes in the adult neurones are one and the same structure. More recently, Parat has interpreted the 'dictyosomes' on the surface of the idiosome (thought by many to be Golgi material) as 'special' chondriosomes, or 'lepidosomes.' He likewise distinguishes in certain other cases 'active' chondriosomes and 'diffuse lipoids.' Still other investigators think that the Golgi apparatus is formed from the mitochondria (Ma, Lim, and Lu, '27).

In view of so much confusion regarding the individuality of these supposed cytoplasmic components, the present study was undertaken in an effort to determine as far as possible the occurrence and genetic relationship of these structures in the islet cells of Langerhans. To anticipate the conclusions of this investigation, it may be said that this study supports the view that the Golgi apparatus, neutral-red bodies (vacuome), and mitochondria are morphologically distinct structures in the islet cells. The canalicular apparatus appears to represent the cytoplasmic mold of which the Golgi material forms the cast (in fixed preparations). The trophospongium is thought to represent the whole or part of the negative image of the Golgi apparatus. It seems probable that Holmgren confused the extensions of the trophospongium to the surface of the cell as figured by him in the islet cells with some exogenous structures.

No attempt will be made here to summarize the enormous literature that has accumulated on the various phases of the general subject. Only those papers to which direct references are made will be listed.

It is a pleasure to express my deep gratitude to Prof. H. E. Jordan for his many helpful suggestions and for the encouragement given me during the progress of this work. I am likewise indebted to Alice Clarke Mullen for her painstaking care in making the drawings.

MATERIAL AND METHODS

The material employed in this study consists of the islets of Langerhans of the pancreas of the albino rat. The details of the methods will be omitted here and the reader is referred to the original papers and the recent summaries by Bowen

('28) of methods for the demonstration of the Golgi apparatus, and to the chapter on cytological methods by Gatenby in Lee ('28).

For the demonstration of the Golgi apparatus in the islet cells of the pancreas, the osmic-tetroxide method of Mann-Kopsch (Weigl) as modified by Ludford ('26) and the Kolatchev method as used by Nassonov ('23–'24) were employed.

To disclose the canalicular apparatus, the potassium-bichromate-formaldehyde hematoxylin method of Regaud ('10) gave good results. The aortic injection of neutral red (1: 10,000) in normal saline after the method of Bensley ('11) also was used. This method reveals faintly clear spaces among deeply stained granules.

Attempts were made to demonstrate the positive of Holmgren's trophospongium by using his trichloracetic-acid fixation and following this with Weigert's resorcin-fuchsin elastic-tissue stain and iron hematoxylin. The method has not proved very successful in my hands. Only in a few cases was the trophospongium only slightly suggested following this technique.

The mitochondria were revealed by the method of Regaud ('10) and at times could be seen very faintly after the injection of Janus green B following the procedure recommended by Bensley ('11).

To demonstrate the vacuome, solutions of neutral red varying from 1 per cent aqueous (or normal saline) to a saturated solution were used. The method of administering the vital dye was essentially that described by Beams ('30) for the acinar cells of the pancreas. However, it is very difficult to recognize the position of the islets among the acinar tissue following the mounting of a small piece of the entire pancreas under the oil-immersion lens. If a portion of the intravitam-stained pancreas is removed, placed in Ringer's solution, and observed under the dissection microscope, the islets can be reasily recognized. The lobules of the pancreas are then teased apart, and the islets segregated and mounted on a slide with Ringer solution and studied under the oil-immersion objective.

To disclose the Golgi apparatus and the vacuome (neutral-red osmiophile bodies) in the same cell of the island of Langerhans, the neutral-red and modified Mann-Kopsch (Weigl) method as used by Beams ('30) was strictly followed. After this technique the reticulated Golgi apparatus and the black granules (neutral-red osmiophile bodies) are discernible in the same cell.

OBSERVATIONS AND CONSIDERATIONS

The Golgi apparatus in the islet cells of the pancreas has been observed by v. Bergen ('04), Bensley ('11), Cajal ('14), Saguchi ('20), Morelle ('25), Bowen ('26), and Ludford and Cramer ('27). A slight variation in the morphological details of the Golgi apparatus has been reported by these investigators. However, the majority are in agreement that it is present in the islet cells in the form of reticulated filaments. Recent work by Ludford and Cramer has disclosed the fact that the Golgi apparatus in the islets of Langerhans is affected by different physiological states of the gland which appear to be directly associated with the phenomenon of secretion. Ludford and Cramer especially have figured marked changes in size and distribution of the Golgi material following exposure of the animal to heat and during the period of gestation.

Figure 1 shows the Golgi apparatus in the islet cells in what appears to be its usual pattern. It presents considerable variation in form in cells of the same islet. The Golgi apparatus is deeply impregnated and gives a sharp contrast with the surrounding cytoplasm. Its filaments are fairly smooth and show no indication that the 'osmiophil canaliculi' have been formed by the fusion of discrete bodies. There is no direct evidence that the Golgi apparatus is limited by a membrane, although this is presumed to be the case.

A condition of especial interest inheres in the apparent absence of polarization of the Golgi apparatus in the islet cells. In this respect the more or less unpolarized position of the Golgi apparatus in the islet cells differs remarkably from the condition found in most other glands of vertebrates. In fact, recent studies by Cowdry ('22) and Ludford and Cramer ('28) on the position of the Golgi apparatus in different physiological states of the cells of the thyroid gland have brought forth convincing evidence that the reversal of secretory polarity takes place concomitant with the reversal in position of the Golgi apparatus. Inasmuch as the secretory phenomena in the islet cells are so little understood and the secretory polarity unknown, the significance of the lack of a definite polarity of the Golgi apparatus remains obscure.

Following application of the modified Mann-Kopsch method, granules (lipoid corpuscles of Saguchi?) are often observed in certain of the islet cells (fig. 1). There appears to be usually only one granule present in a cell, and this frequently gives a dark brown reaction with osmic acid. The significance of these granules is not definitely known, although it would appear probable that they may be associated with the secretory products.

Canalicular apparatus

Figure 2 illustrates the canalicular apparatus as disclosed by the method of Regaud ('10). One is immediately impressed by the general and somewhat detailed resemblance of the distribution and form of the canalicular apparatus to that of the Golgi apparatus (fig. 1). In general, a similar topographical relationship to the nucleus as that shown by the Golgi apparatus is striking. In many cases the canals can be seen extending well along the sides of the nucleus, not unlike the condition often found as regards the Golgi apparatus. Also, the lack of any definite polarization in the cell of the canalicular apparatus is comparable to that of the Golgi apparatus. It is to be expected that slight variations may occur in the morphological details between the appearance of the Golgi apparatus and the canalicular apparatus; these are probably explainable on the basis of the techniques employed. The writer is of the opinion that the canalicular apparatus represents the negative image of the Golgi apparatus, and that it is not induced by mechanical manipulation.

It is of interest to note that Regaud's technique does not disclose the canalicular apparatus in all types of tissues. This fact would seem to suggest that the Golgi material must vary somewhat chemically in cells of different tissues.

In some of the preparations following the method of potassium-bichromate-formaldehyde hematoxylin technique a deep blue color appears bordering the clear canals (fig. 6). The question immediately arises whether this can be interpreted in terms of a 'limiting membrane' plus a more peripheral portion of the Golgi material, or a concentrated area of mitochondria; or whether it might be associated with secretory products, or represent actually a combination of several of the above-named structures. Concerning these possibilities I am inclined to favor the view that this deeply stained area represents a portion of the more peripheral Golgi material plus the 'limiting membrane,' with a variable concentration of mitochondria at its surface. It is entirely reasonable, it seems to me, to assume that a more rigid or gel-like portion of the supposed limiting area of the Golgi material which is in direct contact with the cytoplasm has not been completely dissolved by the reagents of the technique employed. It would appear plausible, then, that the presumed limiting area differs slightly chemically, and perhaps physically, from the remainder of the Golgi material. If, then, one assumes the view that the Golgi apparatus is possibly a duplex system, that is to say, proteid linked with a lipoid, it would appear probable that the 'limiting membrane' is more in the nature of a proteid than of a lipoid.

Clear canals of Bensley

Recent observations by O'Leary on the living islet cells of the mouse confirm the findings of Bensley ('11) on the guinea-pig, namely, that "it is possible to observe in the still living cell the outlines of the canals of Holmgren, which appear as colorless spaces among the deeply stained granules." O'Leary further adds that "The canalicular apparatus of the islet cells does not segregate the dye neutral red when it is applied vitally by appropriate methods." He finds the canals of Holmgren located in the greatest expanse of the cytoplasm, which may appear at the capillary or extracapillary pole of the columnar-type islet cell which apparently abuts at but one pole upon a capillary. He states further that the position of the clear canals is equidistant from the converging streams in those polyhedral cells whose several surfaces are in contact with capillaries.

Bensley, on the other hand, seems always to find the canals of Holmgren located on the pole of the nucleus farthest from the capillaries. He observed, also, that the canals extend well around the nucleus in the direction of the capillaries. Bensley interprets the clear canals, following the neutral-red technique, as similar to those revealed in fixed preparations.

The observations here recorded on islet cells treated according to the neutral-red method of Bensley describe faithfully the figures published by O'Leary (fig. 3). The clear spaces are not stained with neutral red and they often present a complete reticulum under proper conditions. In cells which abut with their base upon a capillary the clear 'canals' are usually located in the area of the cell at the pole of the nucleus opposite the capillary.

It is obvious that the clear 'canals' following the vital technique do not appear in all details to represent the Golgi apparatus or the canalicular apparatus following the modified Mann-Kopsch (Weigl) and Regaud techniques, respectively. They differ morphologically from the above-named structures in being slightly larger in girth in general, and varying in girth considerably throughout their complete or partial reticulum. Furthermore, it is not definitely known whether the clear spaces are really cytoplasmic canals. In any case, they appear to be homogeneous, with about the same refractive index as the surrounding cytoplasm, and they are unquestionably void of neutral-red granules.

It is apparent now that the neutral-red granules of Bensley ('specific granules') are not the same as the vacuome of Parat. They are much more numerous and considerably smaller than the neutral-red bodies of Parat. The 'specific granules' of the islet cells are readily colored by Bensley's method in the rat, and they do not deviate from the description given by Bensley for the guinea-pig and by O'Leary for the mouse. The vacuome is not usually colored in the islet cells of the rat following the neutral-red method of Bensley ('11).

Vacuome

The neutral-red granules (vacuome) appear in the islet cells following the injection of neutral red into the living animal. They are smaller than the neutral-red bodies in the acinar cells, but are considerably larger than the 'specific granules.' The neutral-red bodies of the islet cells are unlike the neutral-red bodies in the acinar tissue in that they appear to lack any marked polarization. They are, however, slightly aggregated in the paranuclear region of the cell. The neutral-red bodies in the islet cells are considerably more difficult to demonstrate than are those of the acinar tissue. Often only a few cells of a whole islet will show the neutral-red bodies. However, in favorable preparations they can be readily demonstrated. They are smaller and more numerous than the 'lipoid corpuscles.' It is assumed that these neutral-red bodies are what Parat would term the vacuome. In any case, they are the only bodies that are colored by neutral red in the islet cells other than the 'specific granules' and at times the 'lipoid corpuscles.' It appears probable to the writer that the neutral-red bodies observed by O'Leary in the intravitam-stained islet cells, and tentatively interpreted by him as 'lipoid corpuscles,' are really the vacuome of Parat.

A point of considerable significance emerges here in that the neutral-red bodies appear to possess no marked relationship to the Golgi apparatus. They seem to be morphologically distinct bodies with no tendency toward alignment or fusion to form a reticulum (Golgi apparatus). The neutral-red bodies are unquestionably distinct from the Golgi apparatus, canalicular apparatus, and clear canals of Bensley.

Here, as in the acinar cells (Beams, '30), the application of the modified Mann-Kopsch (Weigl) technique to tissue previously stained intravitam by neutral red discloses bodies of the same nature as those stained by neutral red alone (fig. 5). There can be little doubt, it seems to me, that the black bodies disclosed after the neutral-red osmic-acid technique represent the vacuome of Parat. It is noteworthy that these bodies (neutral-red osmiophile) are not found in islet cells following osmic-acid and silver-nitrate techniques alone (Bensley, '11; Cajal, '14; Bowen, '26; Ludford and Cramer, '27). However, argentophile granules have been reported by Saguchi ('20) in and between the islet cells of the frog pancreas. He thought that these bodies were derived from nucleoline and eliminated from the cell body or transformed into other cellular constituents. It may be that Saguchi was impregnating the vacuome, but it appears likely from his figures that it was probably something different.

In successful preparations of intravitam-stained vacuome followed by the modified Mann-Kopsch (Weigl) method, the Golgi apparatus is disclosed in the same cell (fig. 5). The evidence, accordingly, appears totally lacking that the Golgi apparatus is formed by the action of osmic acid upon the neutral-red bodies.

Mitochondria

The mitochondria in the islet cells of the pancreas have been described by Bensley ('11), Cajal ('14), Saguchi ('20), Ludford and Cramer ('27), and O'Leary ('30).

The mitochondria appear in the islet cells as short rods and granules (fig. 6). It has been the writer's experience to find that the mitochondria are not so long and filamentous in the islet cells as they appear in the acinar tissue. As pointed out by Ludford and Cramer ('27), different islet cells show considerable variation in form and distribution of their mitochondria. The form and distribution of the mitochondria offer little evidence that they are directly morphologically related to the Golgi apparatus. In this respect it is an interesting fact that the mitochondria can be demonstrated in preparations in which only the negative image of the Golgi apparatus is apparent. Furthermore, the mitochondria are rarely colored in the islet cells following the modified Mann-Kopsch (Weigl) technique. This evidence would seem to indicate that the Golgi apparatus is perhaps not so closely related chemically to the mitochondria as has been often thought. However, it is worthy of note that there are apparently more mitochondria at the surface of the canalicular apparatus than in any other region of the cell.

DISCUSSION

The foregoing observations indicate that there are unquestionably in the cytoplasm of the islet cells of Langerhans, disclosed after appropriate techniques, a Golgi apparatus, a canalicular system, a system of clear spaces (Bensley's canals), mitochondria, neutral-red bodies ('vacuome'), 'specific granules,' and 'lipoid corpuscles' (?).

The observations on these structures are not new, since they have been described in the islet cells by previous investigators. However, it is hoped that the evidence here presented may be useful in attempting a profitable discussion of the morphological relationship that may perhaps exist between the above-named supposed cytoplasmic components.

As pointed out above, our knowledge concerning the real nature and function of the Golgi apparatus has not been definitely established. Numerous attempts to develop an explanation of the nature and function of the Golgi apparatus have been made. It is held by some that the Golgi apparatus is chiefly composed of a lipoidal substance in some way linked with a proteid (Cajal, '14; Gatenby, '20; Bowen, '26, and others). This view is substantiated by the fact that the Golgi apparatus is dissolved in lipoidal solvents and blackens with osmic acid—which reactions together with others suggest that it consists largely of some lipoidal compound. If, then, one accepts the view that the Golgi apparatus is chiefly lipoidal in nature, it is easy to understand how the Golgi material could be dissolved, leaving in the fixed cytoplasm clear spaces marking the position formerly occupied by the Golgi material (canalicular apparatus).

This interpretation offers a plausible explanation of the clear canaliculi (following certain techniques) in the islet cells of the pancreas. Morphologically and topographically, the clear spaces appear to represent quite accurately what one should expect as regards a negative image of the Golgi apparatus. To be sure, there may appear cracks in the cells which give in some instances a deceptive appearance of the negative image of the Golgi apparatus, although careful scrutiny will permit one easily to discriminate between the artifact and the canalicular system. Accordingly, the evidence presented in this paper is in agreement with the prevailing view, namely, that the canalicular system (trophospongium) is merely the negative image of the Golgi apparatus (Bergen, '04; Cajal, '14; Duesberg, '20; Saguchi, '20; Cowdry, '24; Bowen, '28, and others). Duesberg ('20), however, takes certain exceptions to this general statement and is of the opinion that there must be two types of trophospongium.

> The neurones and the non-nervous cells with a localized trophospongium on the one side, and the non-nervous cells with a diffuse trophospongium on the other. As to the latter, the identity of both formations can be rejected without further discussion; for, while the trophospongium extends all over the cytoplasm, the apparatus of Golgi is localized at one pole of the nucleus (exceptions made for lutein cells). As to the former, both formations appeared to me to be identical.

Cowdry ('21) is of the opinion that Duesberg does not give sufficient consideration to the different types of techniques that may perhaps vary the appearance of the cytoplasmic structures, and, furthermore, that the form of the Golgi apparatus is undoubtedly variable in different cells of the same type of tissue. Nevertheless, Holmgren's theory has found strong support in the work of Ross ('15–'25) and Penfield ('22) on nerve cells, in which they think the Golgi apparatus and the trophospongium are quite distinct structures. In fact, Penfield remarks, "There cannot be a positive and a negative picture of the same structure, as has been widely maintained. They are separate structures demonstrable simultaneously." Penfield seems correct in assuming that there cannot be a positive and a negative image of the same material. But I am convinced, from a study of the islet cells, that the canalicular apparatus represents the negative image of the Golgi apparatus. Whether the canalicular apparatus is the same as the trophospongium cannot positively be said. However, the canalicular apparatus and the Golgi apparatus are the only two reticulated structures that I have been able to demonstrate in the islet cells.[1] Then, too, a comparison of the figures of Holmgren ('04) on the islet cells with those of the Golgi apparatus (Cajal, Bowen, Ludford and Cramer, and in this paper) reveals a striking similarity. Therefore, it would seem, as suggested by Jordan ('21), that the Golgi substance is dissolved (following certain techniques), leaving behind the cytoplasmic mold of which the Golgi material formed the cast, and that this negative image of the Golgi apparatus is the same as the trophospongium of Holmgren. By this statement the author does not wish to imply that the Golgi material in the living cell is in any sense a solid cord, as has been held by some.

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[1] Studies in progress indicate that there are in the spinal-ganglion cells clear spaces that are quite distinct from the Golgi apparatus. It appears likely at present that these clear spaces may perhaps be associated with the formation of Nissl bodies.

A more difficult question arises regarding the morphological relationship that apparently exists between the Golgi apparatus and the clear canals observed by Bensley, O'Leary, and the writer following neutral-red techniques. Bensley and O'Leary seem to accept the hypothesis that the clear canals are the canals of Holmgren, which in turn represent the Golgi apparatus. However, if one follows the description of Bensley, he is impressed by the lack of identity in minute details of morphological pattern and topographical relationship between the two. Furthermore, the canalicular apparatus as disclosed by Regaud's method does not precisely duplicate the clear canals (neutral-red method) of Bensley; and such clear reticulated canals of Bensley (neutral-red method) are not often observed in tissues other than the islet and spinal-ganglion cells (Cowdry, '21). Moreover, the clear canals of Bensley observed in the living tissue are subject to considerable variation in girth and continuity of reticulum.

As regards the above-named difficulties in homologizing the clear canals of Bensley with the apparatus of Golgi, one must take into consideration the conditions under which the observations are made. It is perfectly possible, it seems to me, that the Golgi apparatus is of a physical consistency similar to that of the surrounding cytoplasm, in which case compression of the cover-glass upon the living cells for oil-immersion vision might distort the canals, giving a deceptive appearance of its original form. Likewise, the large clear vacuolar spaces sometimes encountered are interpreted on the basis of inadequate preparations, giving only incomplete information of the structural reality.

While the evidence for and against homologizing the clear canals of Bensley and the Golgi apparatus is somewhat uncertain, I am inclined tentatively to accept the interpretation that the canals represent the Golgi apparatus. Since we know that the Golgi apparatus is extremely variable in the islet cells (Ludford and Cramer, '27) and that it is very likely easily distorted mechanically by the application of the cover-glass upon the living tissue, this interpretation seems the more legitimate. The possibility that the clear canals of Bensley might represent some as yet unrecognized system in the cell seems highly improbable. As previously pointed out (Beams, '30), if we accept the view that the canals of Bensley represent the Golgi apparatus, we have a striking case in which the Golgi apparatus may exist as a network in the living cell, but be refractory to coloration by neutral red.

The evidence in the islet cells supports the claim that the Golgi apparatus and canalicular apparatus are not in direct communication with the exterior of the cell, and it substantiates the view that Holmgren must have confused the trophospongium with some exogenous processes which may perhaps in certain cases penetrate the cell.

The theory of Parat and Painlévé has received fairly wide support of very recent date. However, it is apparent that many of the recent investigators have found the vacuome theory untenable as it is applied to the Golgi apparatus. I venture to conclude that most of the recent workers are convinced that a system of vacuoles in animal cells can be demonstrated following certain techniques, but that it still remains an open question whether these bodies are always preformed or secondarily induced. In this connection arises the question of why the vacuome in the acinar and islet cells of the pancreas is not impregnated with osmic acid unless it be previously stained vitally by neutral red. This fact seems to me interpretable in one of two ways: The neutral red may change the content of the vacuoles chemically in such a manner as to permit reduction of the osmic acid; the vacuome is not pre-existing, but induced by the neutral red injected and subsequently blackened by osmic acid. Of these two views, I am inclined to accept the former, although considerable evidence has been developed recently by Chlopin ('27) and Kunio Sato ('30) to support the latter. It is obvious that the final answer to this question awaits further investigations.

Recently, Parat ('28) has introduced two new terms into animal cytology: 'active' chondriosomes and 'diffuse lipoids.'

Both of these designations seem somewhat vague. It is no doubt true that Parat's new methods may reveal the Golgi apparatus at times, just as do many other methods. However, it seems to the writer that if Parat identifies his 'active' chondriosomes with what others have called Golgi apparatus, then the introduction of the term is undesirable; first, because of lack of priority, and secondly, because there appears no good reason for complicating the literature further by the coining of new terms for those that are already fairly well established. If, on the other hand, Parat's 'active' chondriosomes are quite distinct from the Golgi apparatus, mitochondria, or vacuome, then he has no justification, it seems to me, for the comparisons he has made regarding the 'active' chondriosomes, vacuome, and Golgi apparatus.

As pointed out by Gatenby ('29), Parat is due credit for his enthusiasm and persistence in maintaining and establishing his claims for the vacuome. Yet, "he has vitiated much that is good in his work by introducing the theory that such vacuoles are the Golgi apparatus, and endeavoring to prove that the real Golgi apparatus is merely a group of modified mitochondria, his lipidosomes." Then, too, Gatenby points out that the view that the Golgi apparatus and the vacuome are the same structure is disproved by the fact that "the neutral-red staining vacuome is not consistently argentophil, whereas the Golgi apparatus is." A similar condition is suggested regarding the reactions of the Golgi apparatus and vacuome in the islet cells to the treatment with osmic acid. The Golgi apparatus is always osmiophile, whereas the vacuome is not.

Other pointed criticisms of Parat's theory have been made by many, especially by Avel ('25) and Bowen ('27), whose arguments strike at the very foundation of this theory. Inasmuch as the typical net-like structure of the Golgi apparatus and the neutral-red osmiophile bodies (vacuome) can be clearly demonstrated in the same cell (Beams, '30, and in this paper), a serious objection arises to Parat's claims that the Golgi apparatus is formed by action of osmic acid within, between, and on the surfaces of the neutral-red-staining vacuoles and granules in the cell.

SUMMARY

1. The Golgi apparatus in the islet cells of the pancreas of the rat is subject to considerable variation in form and position. Marked polarization of the Golgi apparatus in these cells is lacking. At times bodies apparently comparable to Saguchi's 'lipoid corpuscles' may be observed following osmic-acid technique.

2. Islet tissue prepared by Regaud's ('10) method shows clear canaliculi which suggest a negative image of the Golgi apparatus. The canalicular apparatus (trophospongium) of Holmgren is thought to represent the negative image of the Golgi apparatus. Holmgren presumably confused the extensions of the trophospongium to the surface of the cells figured by him with some exogenous structure.

3. Following the injection of neutral red into the living animal, there appear bodies which are interpreted as the vacuome of Parat. No marked polarization of these bodies is apparent, although they appear to be somewhat aggregated in the paranuclear zone.

4. Intravitam neutral-red-stained islet cells treated with the modified Mann-Kopsch (Weigl) technique retain the neutral-red bodies impregnated a deep black or brown. The Golgi apparatus is likewise disclosed in the same cell. No such bodies as the neutral-red osmiophile ones (vacuome) are observed in the islet cells following the modified Mann-Kopsch (Weigl) technique alone.

5. Following the neutral-red method of Bensley ('11), clear unstained 'canals' are seen among the deeply stained 'specific granules.' These 'canals' are tentatively interpreted as the Golgi apparatus.

6. The mitochondria appear in the islet cells in the form of short rods and granules. They are often observed slightly concentrated on the surfaces of the 'canalicular apparatus.'

7. It is concluded that the Golgi apparatus in the islets of Langerhans is not formed by the fusion of discrete vacuoles that are 'specifically' stained by neutral red, and, furthermore, that the Golgi apparatus is quite distinct from the mitochondria.

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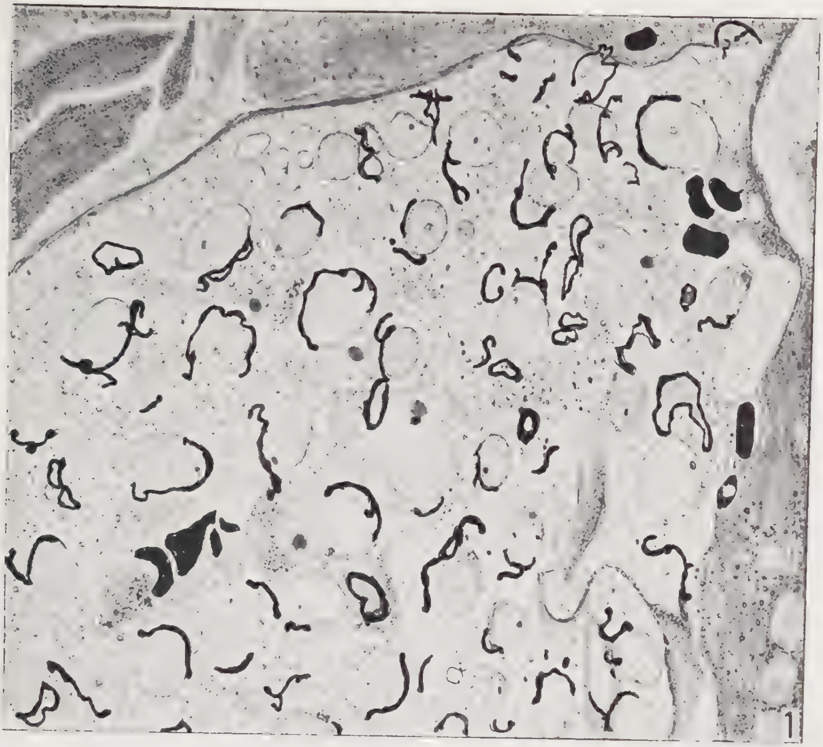
PLATE 1

EXPLANATION OF FIGURES

1 Portion of an islet of Langerhans prepared according to the modified Mann-Kopsch (Weigl) technique. The form of the Golgi apparatus is very variable and unpolarized. Magnification, approximately × 1200.

2 Portion of an islet of Langerhans prepared according to Regaud's potassium-bichromate-formaldehyde hematoxylin technique. Clear spaces are present (canalicular apparatus) which resemble fairly closely the form and position of the Golgi apparatus. The clear spaces are interpreted as the negative image of the Golgi apparatus. The mitochondria are only vaguely shown. Magnification, about × 1200.



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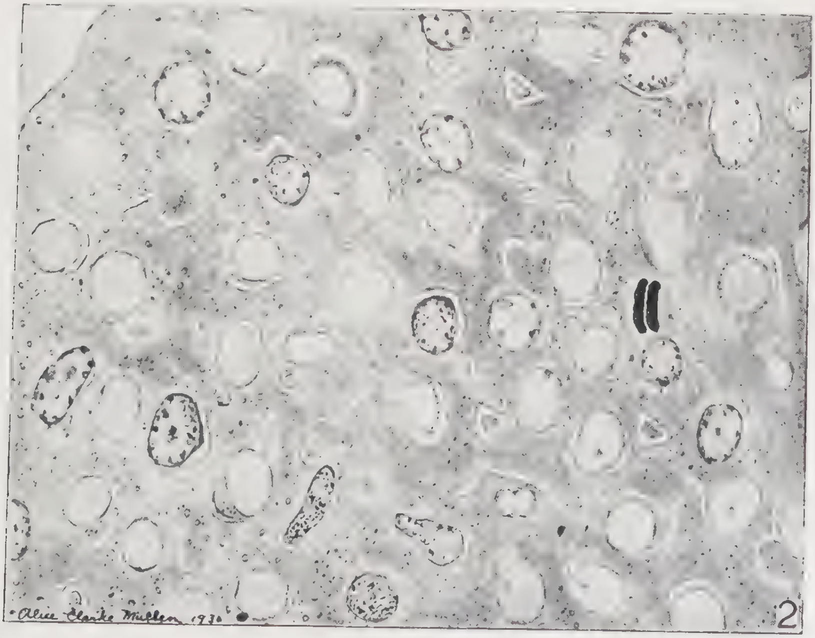
Alice Clarke Mullen 1930

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PLATE 2

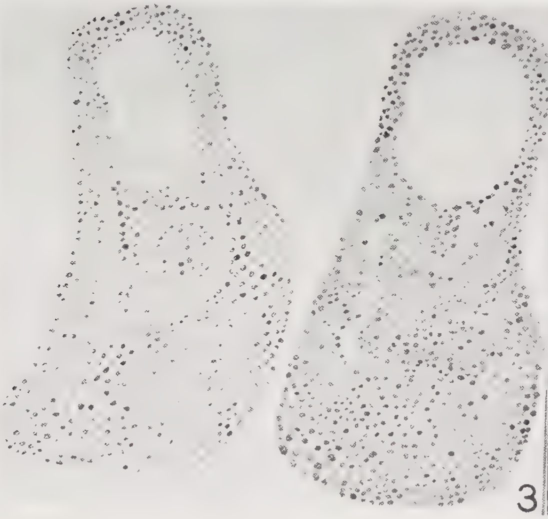
EXPLANATION OF FIGURES

3 Two islet cells greatly magnified to show the 'canals' of Bensley. These clear spaces are faintly, but distinctly, visible among the deeply stained 'specific granules.' Technique, neutral-red method of Bensley ('11). Magnification, about × 3000.

4 Portion of an islet showing neutral-red bodies (vacuome). In some of the cells can be seen faintly the clear 'canals' of Bensley. Technique, neutral-red method as used by Beams ('30). Magnification, approximately × 1400.

5 Section of a portion of an islet showing neutral-red osmiophile bodies (vacuome) and Golgi apparatus in the same cells. They are disclosed as distinct structures. Technique, intravitam stain with neutral red followed by impregnation by modified Mann-Kopsch (Weigl) method. Magnification, approximately × 1400.

6 Section of a portion of islet in which the canalicular apparatus and the mitochondria are disclosed in the same cell. Blue staining occurs around the periphery of the clear canals. The mitochondria seem to be more numerous at the surface of the canalicular apparatus. Technique of Regaud ('10). Magnification, approximately × 1400.



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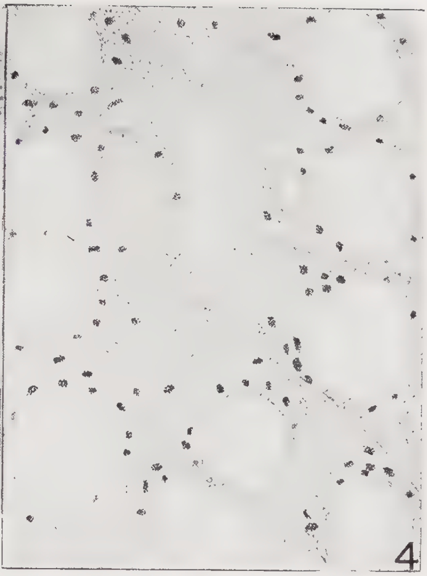

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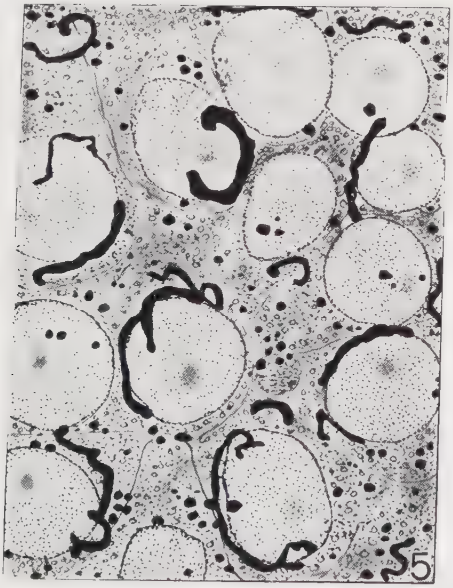

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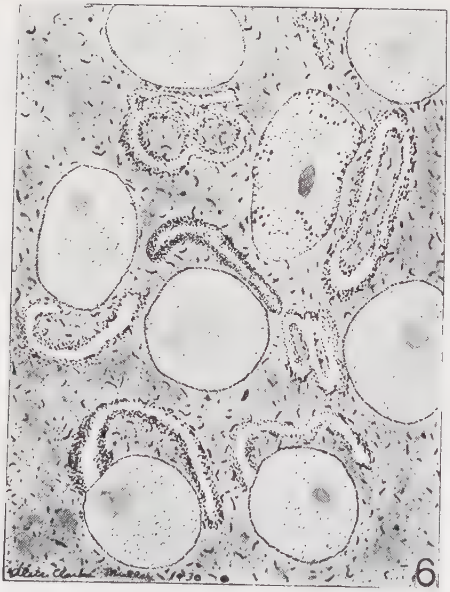
Alice Clarke Murray 1930

6

OCCURRENCE OF VAGINAL PLUG IN A CHIMPANZEE

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ONE FIGURE

Though never before reported in primates, the occurrence of the vaginal plug in rodents has been commonly known since the work of Lataste ('82–'93), who attributed its origin to the seminal vesicle. This conclusion was later verified by Walker, Stockard and Papanicolaou, and Long and Evans, who found that it was a secretory product of the seminal vesicle coagulated by the secretion of a coagulating gland. According to Long and Evans (pp. 72–73)—

> . . . . as sperm are being expelled the secretions of the seminal vesicles and of the coagulating gland mix and evidently 'set' the ejaculated mass, making it adhere tightly to any object with which it is in contact. . . . It is apparent that at the moment of ejaculation the plug is moulded to fit the vaginal lumen, especially at the cervix. . . . The plug adheres so closely to the cornified layer of the vaginal mucosa that it is not easily distinguishable in section. The plug is naturally loosened so that it may be dislodged from the vagina when this cornified layer becomes stripped off spontaneously in every oestrous cycle, as described in an earlier section. [The rodent plug] is not a solid body but contains cavities, especially at its deeper end, these cavities being filled with spermatoza.

In the course of an extended study of the histological and behavioral aspects of the sexual cycle in chimpanzees, which is now under way at the Yale Primate Laboratory, the writer has repeatedly been aware of the presence of a gelatinous mass in the vagina of a mature female chimpanzee when she is continuously in the company of a mature male. The mass is not constantly present, but recurs with each successive receptive period, during which copulation is repeated on an average of three to four times daily. It does not appear when the female is kept separate from the male. The female is receptive during one-half of the thirty-four- to thirty-six-day cycle. The vaginal content is sampled each day by introducing and withdrawing 4 cc. of physiologic salt solution by means of a glass syringe, in the manner used by Hartman in his study of monkeys.[1] Within twenty-four to thirty-six hours after copulation begins, there appear in the daily lavages granules and, at times, small sheets or strands of gelatinous material, of sufficient size and density to obstruct frequently the mouth of the syringe. On the third to fourth day, the diluted vaginal content is usually a mush of gelatinous particles. From that time to the end of the period of receptivity, irregularly shaped strands of the material ranging to a length of 1½ inches are expelled from the vagina, and coitus frequently seems to be painful to the female, in part, apparently, because of the large amount of this substance present.

The first opportunity to determine the general form and extent of the plug occurred in December, 1929. On this occasion the male, in withdrawing after coitus, carried on his still erect penis an enveloping sheet of the gelatinous material. With difficulty, most of this specimen was secured and preserved in neutralized formalin for study. The picture shown herewith was taken with the cooperation of Prof. Alexander Petrunkevitch within a few hours after the plug was secured. During the interval the plug tended to shrink somewhat. The plug was shaped much like the finger of a glove. The cervical end, composed of the three pieces shown at the top of the illustration, was oval, about 15 mm. in diameter, and was solid for a distance of 19 mm. Extending back from this point it became a thin lining of the vagina, except in places where it was deposited in the vaginal folds, as shown by the one long strand which is pictured. The total length of the plug was about 70 mm.

Difficulty was experienced in preparing sections for microscopic study. The gelatinous material crumbled and compressed rather than spread when pressed between slides, making smears of it unobtainable, and it tended to harden when fixed and embedded. Through the cooperation of Prof. Raymond Hussey, excellent serial sections were finally secured. Examination of these showed the substance to be clear and practically structureless, except for the presence of sperm throughout. At the cervical end there were numerous cavities which, as in the case of the rodent plug, were filled with sperm. Microscopic examination of other specimens, discharged from time to time by the same female, has shown that some of them bear an outer layer of epithelial cells and leucocytes from the vaginal wall of the female.

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[1] Hartman, Carl G. 1928. Gestation in a monkey (Macacus rhesus) and associated phenomena. Amer. Jour. Obst. and Gyn., vol. 15, pp. 534–540.

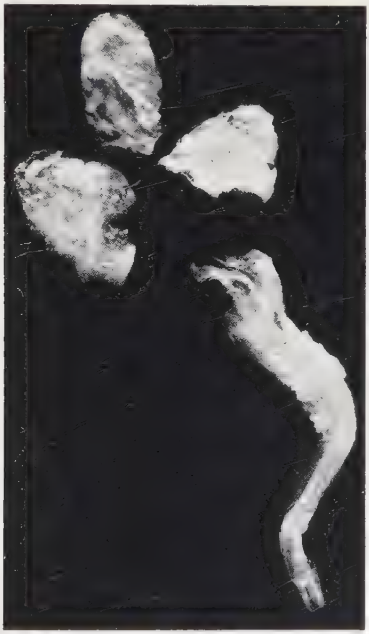

Fig. 1 Vaginal plug from chimpanzee. (Natural size.) The original plug was shaped like the finger of a glove. The three top sections formed the solid tip of the finger near the cervix. The narrow strand was a part of the thin lining of the vagina extending caudad.

The composition of this plug is not to be confused with the cheesy material commonly found in vaginal smears of both rodents and monkeys. That material, as the name suggests, is a dense opaque substance of a yellowish color, which, as Corner points out, is easily identified as consisting of masses of cornified cells. The writer has frequently observed this cheesy substance in monkeys, but under conditions the same as those maintained for the chimpanzees, monkeys have shown nothing analogous to the vaginal plug.

Both the female chimpanzee from whom plugs have been secured and her male mate are fully mature, vigorous animals, apparently normal in every respect. While we cannot be certain that the presence of the plug is typical for chimpanzees until other individuals have been observed, we have no reason to suppose that such is not the case. On the other hand, it is possible that equally frequent and abundant deposits of the male secretions might result in the formation of similar plugs in some of the other primates.

The function of the rodent plug, according to Long and Evans, "apparently is to confine spermatozoa between itself and the uterus as though to give abundant opportunity for them to make their way into the uterus." If a function is to be attributed to this structure, it would appear that, due to the apparent similarity of form and composition, it would serve the same purpose in the chimpanzee. The chimpanzee plug seems similar to that of the rodent in every respect, except in that it is formed from successive depositions of male secretions rather than from one, and, as Long and Evans point out, in the rat sometimes two or even three plugs remain in the vagina at the same time.

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A COMPARATIVE STUDY OF THE 'UTRICULO-ENDOLYMPHATIC VALVE' IN SOME OF THE COMMON MAMMALS

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FOUR PLATES (SIXTEEN FIGURES)

INTRODUCTION

In an article published in The Anatomical Record, September, 1928, Bast described a structure in the human fetal ear which he has named the utriculo-endolymphatic valve. He described it as a highly cellular connective-tissue valve-like flap covered by a columnar epithelium which guards the opening of the utriculo-endolymphatic duct into the utricle.

Yamasaki, in an article published in a Japanese journal in November of the same year, described an area in the wall of the utriculus of the Japanese bat directly opposite the utriculo-endolymphatic valve which he called a macula neglecta of Sarasini. Although his photomicrographs show the valve beautifully, he makes no note of it in his paper.

Wilson and Anson, in July of the following year (1929), published a description of this valve as found in a two-year-old child. Their description was partly based on a model of the valve.

In a later edition of the same year, Anson and Wilson published a second paper on the same structure as found in a number of adult human ears, thus confirming it definitely as a permanent feature of the membranous labyrinth in man.

In 1929, Hoffman and Christensen, in a thesis presented for a bachelor of arts degree, gave a description of the valve as found in the pig. Since then we have collected and studied the ears of rodents, ungulates, carnivores, and primates.

It is the purpose of this work to compare and establish the presence of the utriculo-endolymphatic valves in mammals. This description is based upon a study of the serial sections through these valves and upon wax-model reconstructions of four of them.

MATERIALS AND METHOD

The material used was obtained from animals of varying ages. The ages are as follows: monkey, one and one-half years postnatal; cow, eight months prenatal; sheep, seven months prenatal; pig, sixty-five days prenatal; dog, seven days postnatal; cat, two days postnatal; rabbit, one day postnatal; gray squirrel, adult; guinea-pig, adult; rat, two days postnatal; and gopher, adult.

The celloidin method of embedding was used. The ears were previously fixed in formalin and decalcified in a 5 per cent nitric-acid solution in 70 per cent alcohol. The tissues were then blocked and cut in serial sections 25 μ thick, stained in haematoxylin and eosin, and mounted in series.

Some difficulty was experienced in getting sections which were cut through the full longitudinal extent of the utricular duct and valve. Several series had to be run in several cases before the proper orientation was obtained. In the dog, sheep, and guinea-pig we were unable to obtain the desired orientation, but a study of serial reconstruction showed that the valve was present in all of them. Photomicrographs of the satisfactory cuts are shown in figures 1 to 8.

The valves of the monkey, cow, cat, and gopher were modeled by the wax-plate method, from tracings made with a Bausch & Lomb projection lantern at a magnification of 160 diameters. A 4-mm. wax plate was used to represent each section. In each case as many plates were used as was required in a model of the full width of the opening of the duct into the utricle, the number of sections varying in each case.

Since the models are difficult to photograph, sketches of two views of each model were made. One shows the entire models with the slit-like opening of the utriculo-endolymphatic duct into the utricle. The models were then split along the long axis of the utricular duct and a set of drawings was made of the cut-surface views.

DESCRIPTION

In describing the utriculo-endolymphatic valve it becomes necessary to show its relationship to the utriculo-endolymphatic duct. It can be seen from the cross-sectional photomicrographs and the models that the sacculo-endolymphatic duct at its junction with the utriculo-endolymphatic duct is insensibly continued forward in a horizontal plane as the common endolymphatic duct to the endolymphatic sac.

The utriculo-endolymphatic duct in all the ears studied comes off at an obtuse angle to the common endolymphatic duct and at an acute angle to the sacculo-endolymphatic duct. Coming off in this way, it swings upward around the curved medial wall of the utricle and becomes attached to it by the intervening perilymphatic tissue. Sections through the longitudinal extent of the duct are shown in figures 1 to 8, inclusive. In these photomicrographs the duct is separated from the utricle by two layers of epithelial cells interposed by a connective-tissue layer. This layer is loose-meshed and vascular in the center of the fold, but toward the epithelial walls of both the utricle and duct it becomes more concentrated and adult in nature.

The long scoop-like fold (*UV*) in figures 1 to 8, inclusive, represents the valve cut through its longitudinal extent. In the model drawings in figures 9 to 16, inclusive, this valve-like structure presents a dovetail-like broadening of the relatively narrow utriculo-endolymphatic duct at its junction with the utricle to form a broad, curved, slit-like opening. The amount of curvature in this slit seems to vary in the group of animals studied. It is very pronounced in the cow, but straight in the monkey. In the human the slit-like opening is straight. The ears of the smaller animals studied, also, seemed to have straighter slit-like openings. The thickness of the valvular fold varies with the size of the animal. The larger animals, such as the monkey and cow, have a large amount of intervening connective tissue between the two epithelial layers in comparison to the small amount present in the smaller animals, such as the gopher and rat. As can be seen from the illustrations, the length of the duct varies in the different animals.

Histologically, the structural elements of the flap are the same in all these animals as was described for the human ears, the epithelium varying from a low columnar on the utriculo-endolymphatic-duct aspect to a cuboidal type of epithelium on the utricular aspect of the fold.

In the ear of the cow the medial wall of the utriculo-endolymphatic duct opposite the valve appears glandular because of the folds presented by this side of the wall. Here the epithelium is columnar and lines the crypt-like pockets. This is the area which Yamasaki described as the macula neglecta of Sarasini in the ear of the Japanese bat. In the cow, however, it is a gland-like area and no indication of a sensory epithelium can be noted. In the other mammals this region is a thin wall without any indication of modification.

DISCUSSION

The constant presence of the utricular valve-like fold in the ears of these mammals proves definitely the presence of such a structure in the mammalian membranous labyrinth and strengthens the probability of its functional use.

In an effort to solve some of the mystery surrounding the possible function of this valvular flap the authors worked on the ears of a group of live rabbits.

Attempts were made in this laboratory to inject the membranous labyrinth of the rabbit under as nearly as possible normal conditions, in order to determine the function of the valve. If the utricular fold functions as a valve, it would prevent the pigment passing from the utricular side of the endolymphatic system through the ducts into the saccule and the cochlear endolymphatic portions of the system, whereas if the structure is not functional as a valve, it will allow the material to be dispersed throughout the entire system. The attempted injections were made by way of the ampulla, semicircular canals, or directly into the utricle, a second hole being made to avoid any increase of pressure in the membranous labyrinth. However, when the ears were sectioned later, it was found that the perilymphatic spaces were injected. The very flexible walls of the endolymphatic structures had apparently pushed ahead of the fine capillary needle. It appears extremely essential that some experimental method be devised for successful injection of the vestibular apparatus in order to obtain a clue as to the importance or function of this valve-like fold.

CONCLUSION

From this work we conclude that the presence of the utriculo-endolymphatic valve is a definite and constant feature of the membranous labyrinth of the mammalian ear.

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PLATES

PLATE 1

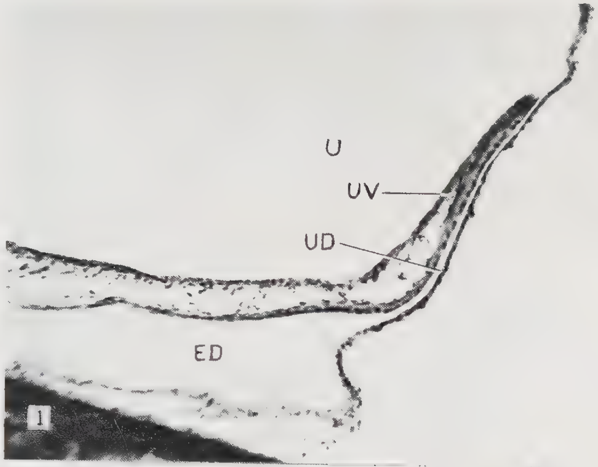
EXPLANATION OF FIGURES

Photomicrographs of sections through the utriculo-endolymphatic valve of:

1 Monkey; age, one and one-half years.
2 Cow; fetus of eight months.
3 Cat; age, two days.
4 Rabbit; age, one day.

ABBREVIATIONS

U, utriculus
S, sacculus
UV, utriculo-endolymphatic valve
UD, utricular duct
ED, endolymphatic duct



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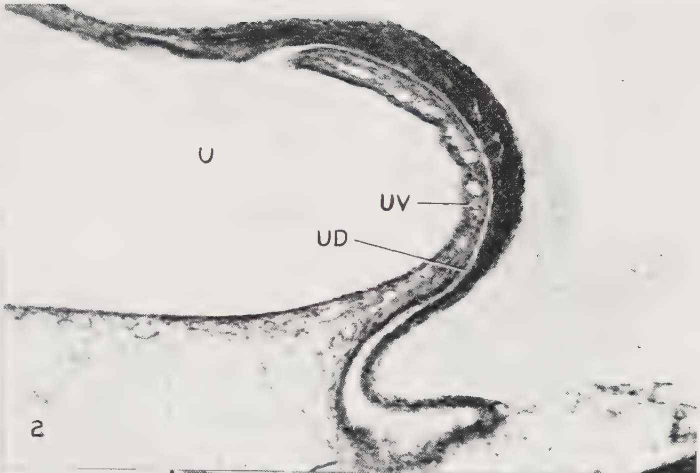

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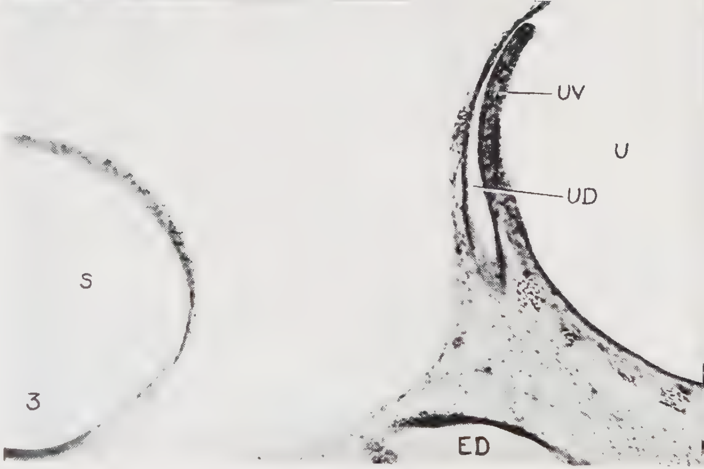

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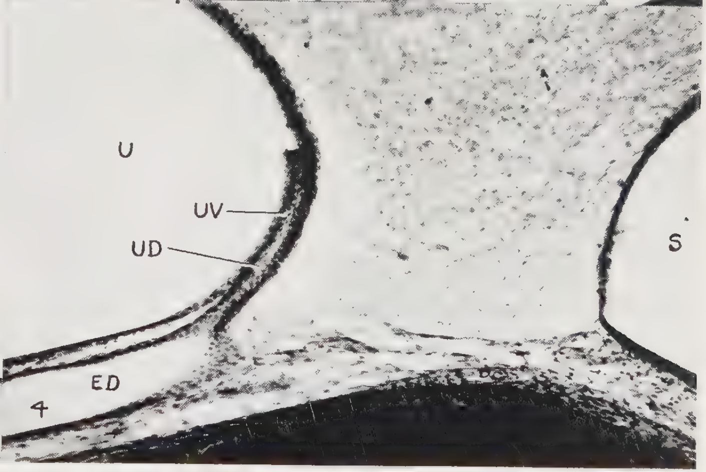

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PLATE 2

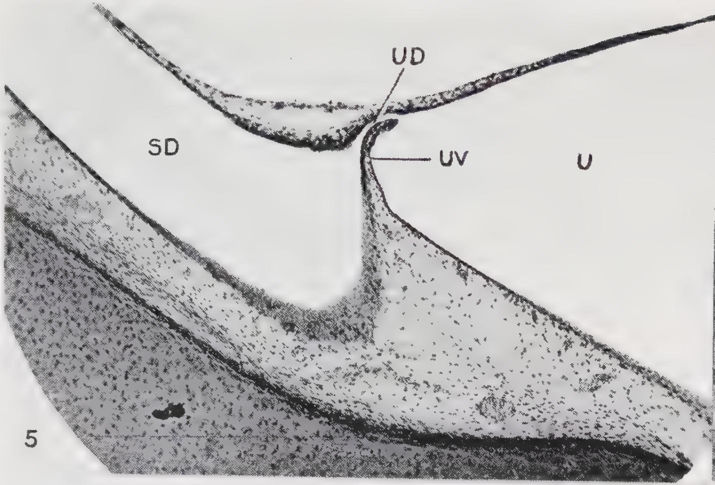
EXPLANATION OF FIGURES

Photomicrographs of sections through the utriculo-endolymphatic valve of:

5 Pig; fetus of three and one-half months.
6 Gray squirrel; adult.
7 Gopher; adult.
8 Rat; age, two days.

ABBREVIATIONS

U, utriculus
S, sacculus
UV, utricular valve
UD, utricular duct
ED, endolymphatic duct
SD, saccular duct



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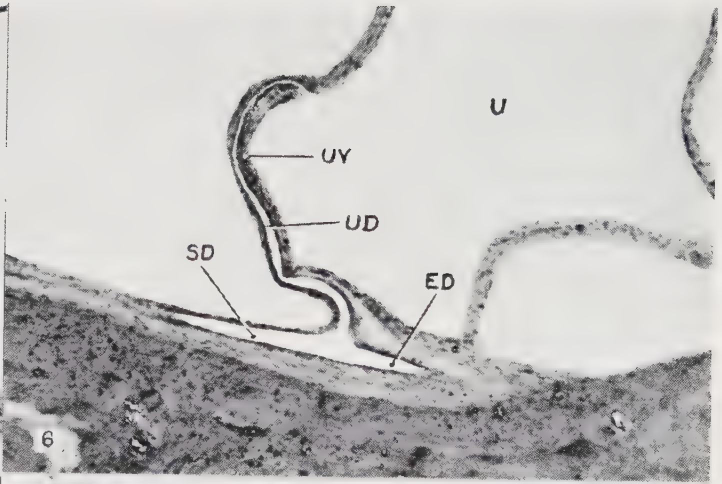

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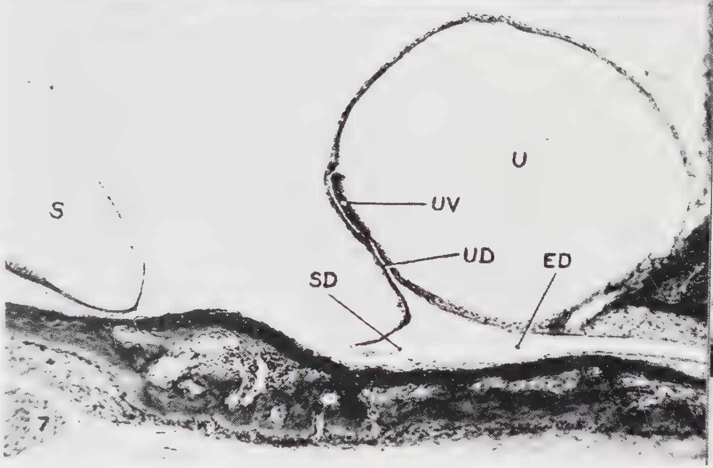
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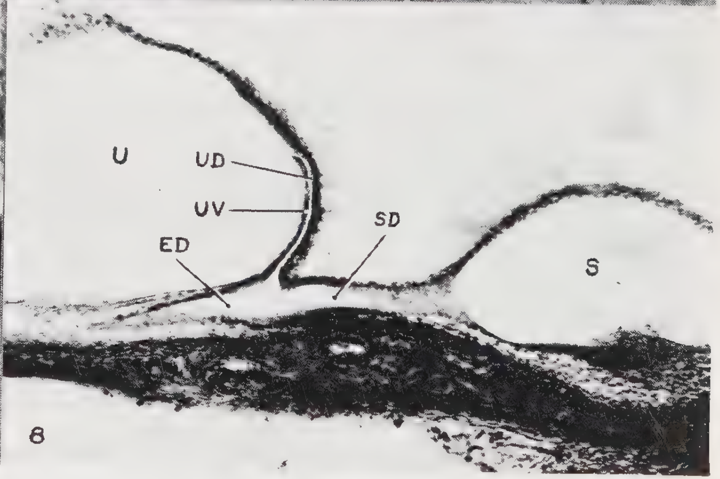
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PLATE 3

EXPLANATION OF FIGURES

Drawings of wax-model reconstructions of the utricular duct and valve region of the monkey and cow.

9 Monkey; one and one-half years old.

10 Same model as 9, but cut open to show extent of utricular duct and valve.

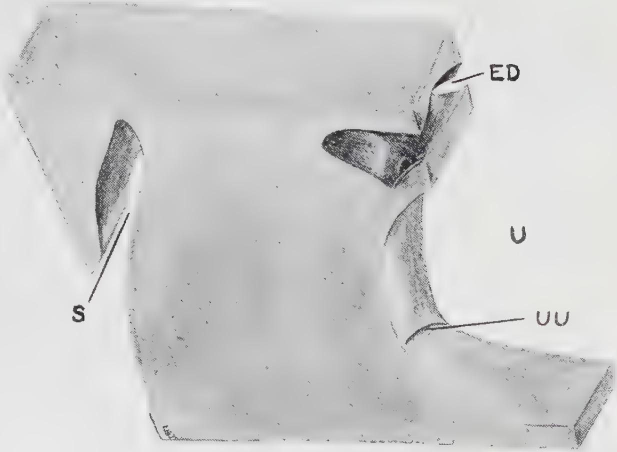
11 Cow; fetus of eight months.

12 Same model as 11, but cut open to show extent of utricular duct and valve.

ABBREVIATIONS

S, sacculus
U, utriculus
ED, endolymphatic duct
UD, utricular duct
UV, utricular valve
UU, slit-like opening of utricular duct into utriculus

PLATE 3



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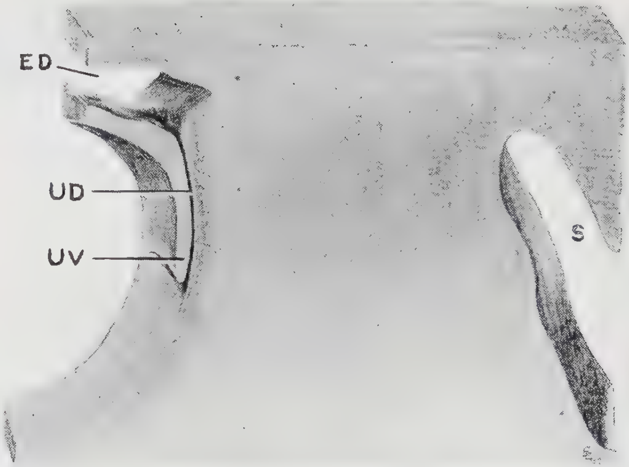

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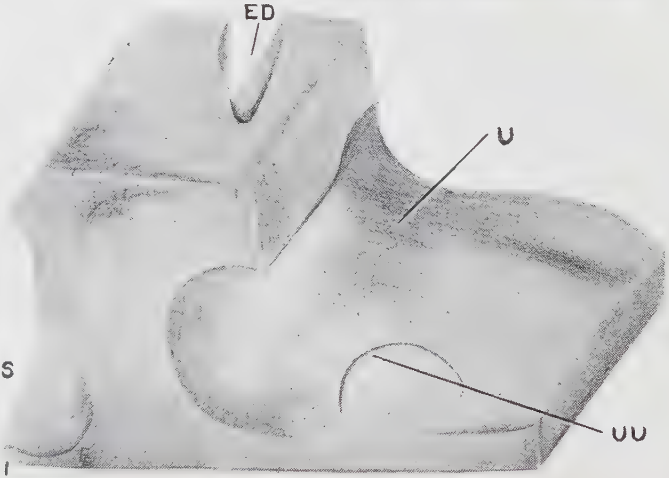

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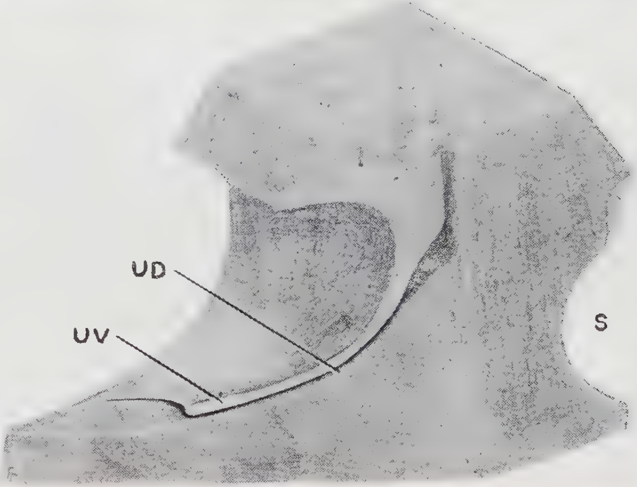

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PLATE 4

EXPLANATION OF FIGURES

Drawings of wax-model reconstructions of the utricular duct and valve region of the cat and gopher.

13 Cat; age, two days.

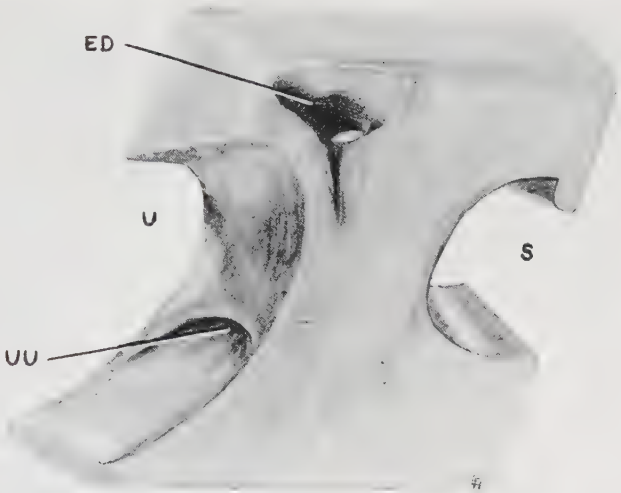
14 Same model as 13, but cut open to show extent of utricular duct and valve.

15 Gopher; adult.

16 Same model as 15, but cut open to show extent of utricular duct and valve.

ABBREVIATIONS

U, utriculus
S, sacculus
ED, endolymphatic duct
UD, utricular duct
UV, utricular valve
UU, slit-like opening of utricular duct into utriculus
SD, saccular duct
VE, opening of utricular duct into endolymphatic duct



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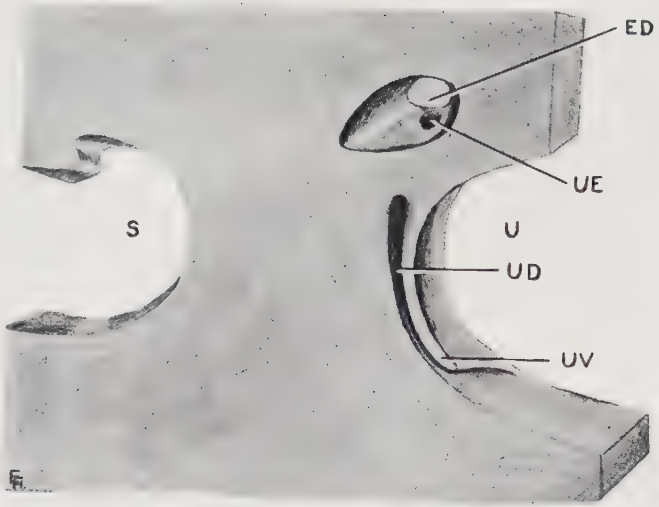
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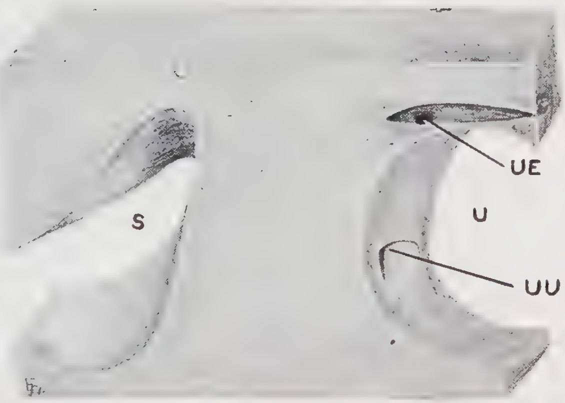

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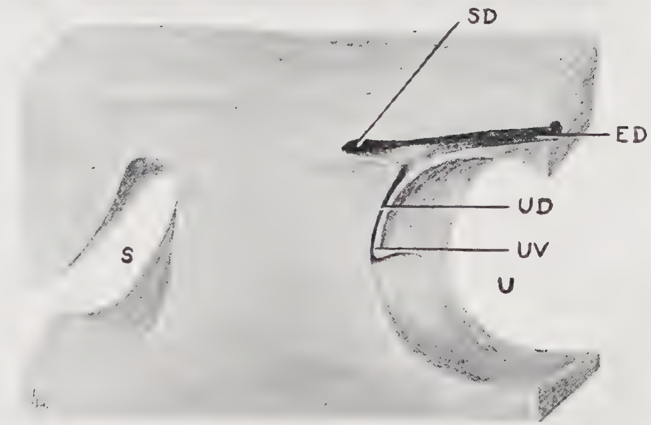
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16

THE GEOMETRIC RELATIONSHIPS OF THE FRONTAL FONTANELLE IN INFANCY

RICHARD E. SCAMMON AND FRED L. ADAIR
Department of Anatomy and Institute of Child Welfare, University of Minnesota

SIX FIGURES

The postnatal involution of the great or frontal fontanelle in man is of considerable clinical as well as scientific interest and a number of quantitative studies have been made of the process. The determination of the magnitude of the fontanelle has been made both by measurements of its diameters and of its area. Measurements of area of the fontanelle are few; in fact, the only extensive published series is that of Nikiforoff ('00), which appeared in an almost inaccessible Russian thesis. The great majority of measurements are of diameters of the fontanelle. Many of the earlier determinations and a number of the later ones are of the longitudinal and transverse diameters of the space along the axes of the sagittal and coronal sutures. It was soon recognized that these measurements were quite unsatisfactory, for it is difficult to determine exactly where the fontanelle ends and the suture begins and the varying shape and extension of the angles of the fontanelle make these particular dimensions poor indicators of the area they are supposed to measure. Many years ago, Elsässer ('43) suggested that a better measurement could be obtained by measuring the two oblique diameters of the spaces (from the apex or summit of the fontanelle margin of one frontal bone to the corresponding marginal apex of the parietal bone of the opposite side) and taking the mean of the two. In practice this measurement, which is commonly called Elsässer's diameter, has been found

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fairly satisfactory, and the majority of modern quantitative studies of the involution of the fontanelle include it.

The problems that we have tried to solve in this study are: What relation does 'Elsässer's' or the mean oblique diameter bear to the area of the fontanelle? How constant is this relation and is it modified with the changing area of the space as it undergoes involution? Does this relationship show any consistent agreement with similar 'rational' relationships deduced from a study of simple geometric figures approximating the form of the frontal fontanelle?

MATERIAL AND TECHNIQUE

The material consisted of measurements of the diameters and areas of the frontal fontanelles of 325 infants ranging from one day to eighteen months in age. The records were obtained from the maternity wards of several Minneapolis hospitals, from the Minneapolis Infant Welfare Stations, and from infants seen in private practice. The last class was limited to young babies brought in for a 'check-up' a few weeks after birth. All these children were full-term, healthy infants. All individuals with manifest clinical evidence of rickets or hydrocephalus were rejected. Otherwise there was no conscious selection of material. The observations were made with the following technique: The child's head was held by an assistant, and an observer passed a strip of thin but closely woven linen, about 20 cm. long and 6 cm. wide, over the sagittal arc of the cranium from glabella to occiput. While this strip was held firmly in place the fontanelle was palpated through it and the fontanelle outline traced upon it with a soft indelible pencil. The record was completed by noting the child's name, age, sex, weight, total or crown-rump length, and horizontal head circumference on the strip. After a little practice, the process was found to be a fairly accurate one. Examples of tracings obtained by this method are shown in figure 1. The areas of the fontanelle tracings were obtained by use of a standardized Amsler polar planimeter. Each determination of area was repeated independently by two persons and the means of the observations used in further computations. Readings were taken to the nearest 0.1 sq.cm. The oblique or Elsässer's diameters were drawn on the tracings with a transparent celluloid straight edge and measured with a steel rule graduated in millimeters and reading to 0.5 mm.

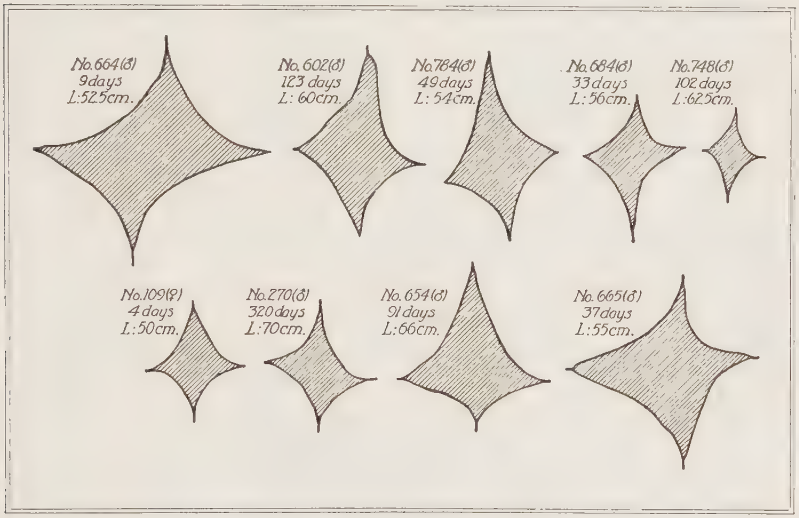
No.664(♂)
9days
L:52.5cm.
No. 602(♂)
123 days
L: 60cm.
No.784(♂)
49days
L: 54cm.
No.684(♂)
33days
L: 56cm.
No.748(♂)
102 days
L:62.5cm.
No.109(♀)
4 days
L:50cm.
No.270(♂)
320days
L:70cm.
No. 654(♂)
91 days
L:66cm.
No. 665(♂)
37days
L:55cm.

Fig. 1 A series of typical tracings of the outlines of the frontal fontanelles of infants in the first year. *L*, total body length in centimeters. (One-half natural size.)

SUMMATION OF DATA

Figure 2 shows the distribution of these data when the observed fontanelle area is plotted against the mean oblique fontanelle diameter. The individual observations are indicated by solid dots. Table 1 shows these observations summed by 0.5-cm. intervals of fontanelle diameter. The means of this relationship progress in a regular fashion, although the individual variation is great. The standard deviations for the various diameter intervals range from 0.2 to nearly 2.3 sq.cm. and the coefficients of variation from about 15 to about 30 per cent. It should be pointed out that both of these measures of dispersion magnify the variability of the material somewhat, since they are for means of considerable intervals of fontanelle diameter and therefore include an apparent variability due to progression as well as the 'true' dispersion of the material.

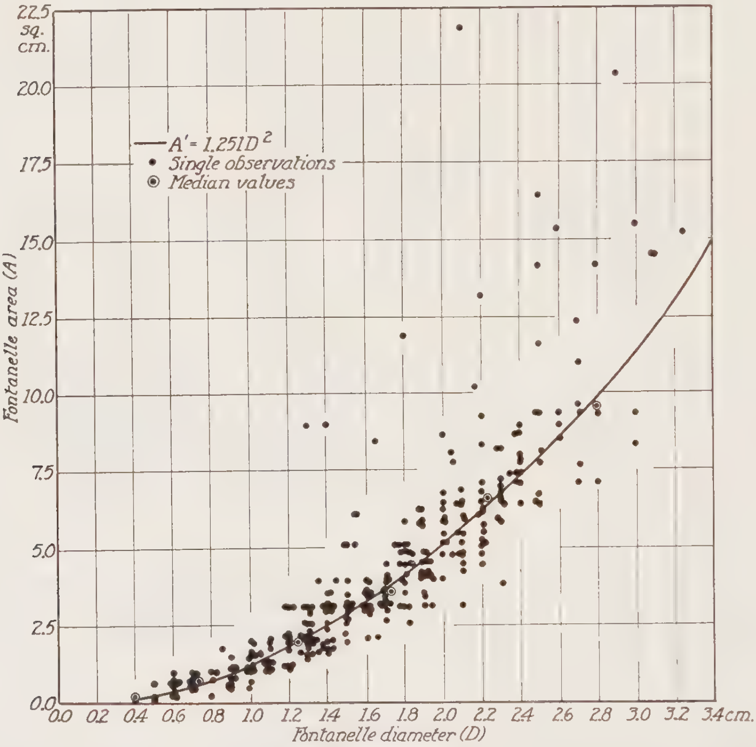

Fig. 2 A field graph showing the distribution of observations on fontanelle area and mean oblique (Elsässer's) diameter of the frontal fontanelle in infancy. Individual observations indicated by dots; medians for 0.5-cm. intervals of fontanelle diameter indicated by circled dots. The curve represents the expression: $A = 1.251\ D^2$.

The degree of scatter of the observations as well as the obvious skewness of this dispersion makes it desirable to apply the percentile method of expression to the material as well as the more accepted one given above. The results of this application are also shown in table 2. These include the median values and semi-interquartile ranges for each 0.5-cm.

TABLE 1

Mean fontanelle diameter and mean fontanelle area
(325 observations)

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		NUMBER OF OBSERVATIONS
Interval	Median	Mean and P.E.	P.E. of distribution	
0.0 to 0.5	0.400	0.190 ..	..	1
0.5 to 1.0	0.734	0.748 ± 0.037	0.227	38
1.0 to 1.5	1.225	2.126 ± 0.077	0.718	87
1.5 to 2.0	1.710	4.051 ± 0.095	0.923	95
2.0 to 2.5	2.189	6.693 ± 0.187	1.612	74
2.5 to 3.0	2.627	10.428 ± 0.466	2.283	24
3.0 to 3.5	3.092	12.904 ± 0.870	2.132	6

TABLE 2

Median fontanelle diameter and median fontanelle area
(325 observations)

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		NUMBER OF OBSERVATIONS
Interval	Mean	Median and S.Q.	Semi-interquartile range	
0.0 to 0.5	0.400	0.19 ..	..	1
0.5 to 1.0	0.725	0.67 ± 0.03	0.19	38
1.0 to 1.5	1.250	2.00 ± 0.05	0.50	87
1.5 to 2.0	1.730	3.67 ± 0.07	0.65	95
2.0 to 2.5	2.230	6.58 ± 0.11	0.92	74
2.5 to 3.5	2.800	9.58 ± 0.39	2.14	30

TABLE 3

Observed mean fontanelle area and fontanelle area calculated from the expression $A = 1.24\ D^{2.12}$

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		DIFFERENCES BETWEEN (a) AND (b)		RATIO OF DIFFERENCE TO P.E. OF OBSERVED MEAN
Interval	Mean	Observed mean (a)	Calculated value ($A = 1.24\ D^{2.12}$) (b)	*Sq.cm.*	*Per cent*	
0.0 to 0.5	0.400	0.190	0.180	+0.010	+ 5.55	..
0.5 to 1.0	0.734	0.748	0.656	+0.092	+14.02	2.50
1.0 to 1.5	1.225	2.126	1.953	+0.173	+ 8.86	2.25
1.5 to 2.0	1.710	4.051	3.974	+0.078	+ 1.95	0.82
2.0 to 2.5	2.189	6.693	6.723	—0.307	— 0.46	0.16
2.5 to 3.0	2.627	10.428	9.914	+0.514	+ 5.18	0.91
3.0 to 3.5	3.092	12.904	14.028	—1.124	— 8.01	0.77

Computations of differences and ratios made from values carried one place farther than given in this table. Number and distribution of observations as given in table 1.

interval as well as a measure called S.Q., which is the semi-interquartile range divided by twice the square root of the number of observations. The S.Q. is in a way comparable with the probable error of the mean, although a less reliable measure, at least for any small sample.

The medians, like the means, of fontanelle area form a decidedly regular progression with respect to the median diameters. But the skewness of the distribution quite evidently affects their magnitudes, which are notably less than the corresponding means. This marked skewness leads us to the opinion that, in spite of all efforts to the contrary, we have probably included in our series a certain number of observations, possibly twenty of the series of 325, that may represent fontanelles affected by rickets or some other pathologic cause. In these cases the enlargement of the fontanelles takes place mainly by extensions along the sutures, and consequently the area is increased without a concomitant increase in fontanelle diameter.

THE EMPIRICAL ANALYSIS OF THE DATA

If the logarithms of the means of fontanelle area are plotted against the logarithms of the means of the corresponding diameters, the resulting distribution is approximately rectilinear and may be represented fairly well by the expression:

$$\log A = \log a + \log D \cdot b \tag{1}$$

where A is the area of the fontanelle, D is the mean oblique diameter, and a and b are empirical constants. This relation is equivalent to

$$A = aD^{b} \tag{2}$$

Determining the constants of this equation by the method of averages, we obtain:

$$A = 1.24\, D^{2.12} \tag{3}$$

The root mean-square deviation of the values calculated by this expression from the observed mean values given in table 3 is 0.34 sq.cm. The weighted mean absolute deviation is 0.20 sq.cm. and the corresponding weighted mean relative deviation is 7.59 per cent. The ratios of the differences between the calculated and observed values to the probable errors of the observed values range from 0.16 to 2.50, the unweighted average ratio being 1.235. On the whole, then, the fit of this expression to the observations upon which it is based may be regarded as a fairly good approximation.

TABLE 4

Observed median fontanelle area and fontanelle area calculated from the expression $A = 1.24\ D^{2.12}$

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		DIFFERENCES BETWEEN (a) AND (b)		RATIO OF DIFFERENCE TO S.Q. OF MEDIAN
Interval	Median	Median (a)	Calculated value ($A = 1.24\ D^{2.12}$) (b)	*Sq.cm.*	*Per cent*	
0.0 to 0.5	0.400	0.19	0.18	+0.01	+ 5.56	..
0.5 to 1.0	0.725	0.67	0.63	+0.04	+ 6.35	1.33
1.0 to 1.5	1.250	2.00	1.99	+0.01	+ 0.50	0.20
1.5 to 2.0	1.730	3.67	3.96	—0.29	— 7.32	4.14
2.0 to 2.5	2.230	6.58	6.79	—0.21	— 3.09	1.96
2.5 to 3.5	2.800	9.58	11.00	—1.42	—11.91	3.63

Number and distribution of observations as given in table 2.

TABLE 5

Observed mean fontanelle area and fontanelle area calculated from the expression $A = D^2$

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		DIFFERENCES BETWEEN (a) AND (b)		RATIO OF DIFFERENCE TO P.E. OF OBSERVED MEAN
Interval	Mean	Observed mean (a)	Calculated value ($A = D^2$) (b)	*Sq.cm.*	*Per cent*	
0.0 to 0.5	0.400	0.190	0.160	+0.030	+18.75	..
0.5 to 1.0	0.734	0.748	0.539	+0.210	+38.90	5.70
1.0 to 1.5	1.225	2.126	1.500	+0.625	+41.68	8.14
1.5 to 2.0	1.710	4.051	2.924	+1.127	+38.54	11.89
2.0 to 2.5	2.189	6.693	4.792	+1.901	+39.67	10.16
2.5 to 3.0	2.627	10.428	6.901	+3.527	+51.10	7.57
3.0 to 3.5	3.092	12.904	9.560	+3.344	+34.97	3.84

Computations of differences and ratios made from values carried one place farther than given in this table. Number and distribution of observations as given in table 1.

THE GEOMETRIC ANALYSIS OF THE DATA

The great fontanelle is often described as a diamond-shaped area or parallelogram, and it seems to be frequently assumed, at least tacitly, that it is essentially a square. Were this the case, the area would be the square of Elsässer's diameter. Table 5 compares the value of D^2 at the mean for each interval of 0.5 cm. of fontanelle diameter in this series with the observed areas for this mean. Obviously, this convention is practically worthless. The observed values are

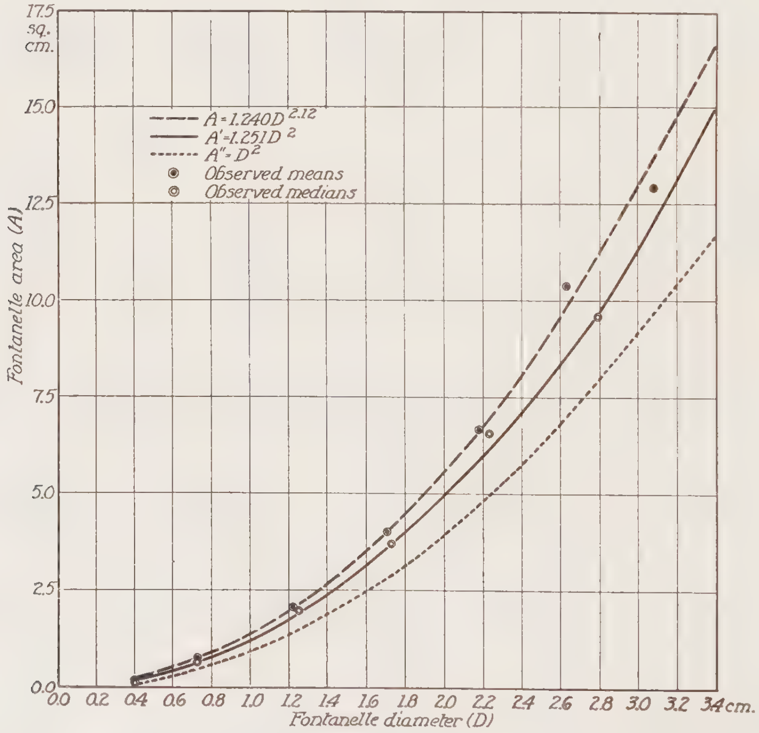

Fig. 3 Measures of central tendency and curves of expressions for the relation between the mean oblique (Elsässer's) diameter of the fontanelle and the fontanelle area in infancy. The observed means of fontanelle area for 0.5-cm. intervals of fontanelle diameter are shown in circled solid dots. The observed medians of fontanelle area for the same intervals are shown in circled open dots. The curve drawn in broken line represents the expression: $A = 1.24\ D^{2.12}$. The curve drawn in solid line represents the expression: $A = 1.251\ D^2$. The curve drawn in dotted line represents the expression: $A = D^2$.

approximately 40 per cent greater than the computed ones and the ratios of the differences of the two values to the probable errors of the observed means range from 3.8 to 11.9. They average 8.51.

A second geometrical approximation may be suggested. This is that the area of the fontanelle may be regarded as that enclosed by the arcs of four equal and tangent circles. In this approximation we regard each fontanelle margin, of

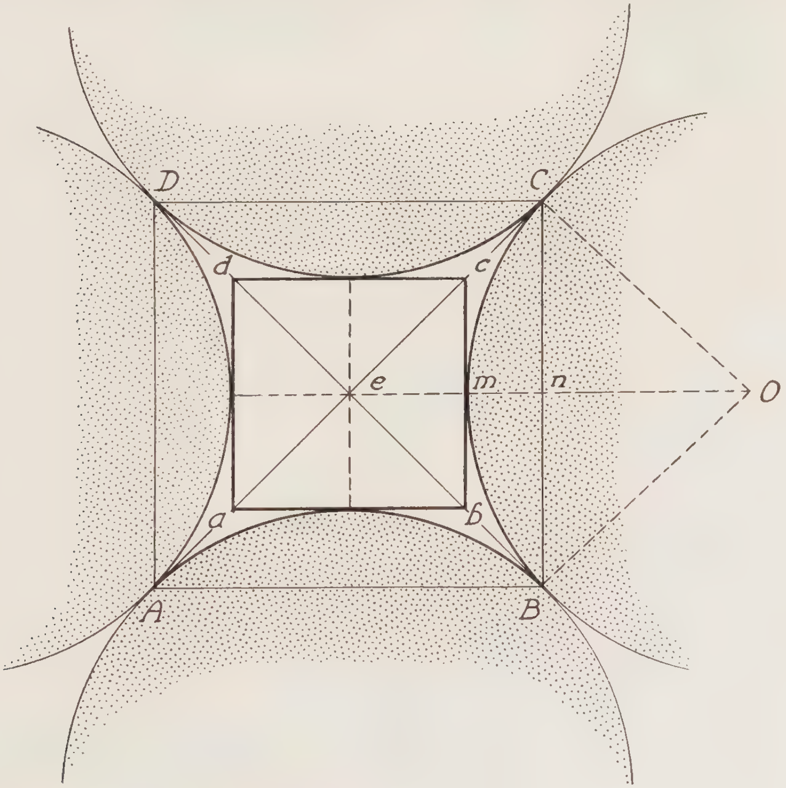

Fig. 4 A construction illustrating the development of a geometric expression for the relation between the mean oblique (Elsässer's) diameter of the frontal fontanelle and the area of the frontal fontanelle in infancy. The shaded circles represent the four bordering bones of the fontanelle (right and left frontal and right and left parietal bones). *A, B, C,* and *D* represent the points of contact of these bones and the angles of the fontanelle. The lines *ac* and *db* represent the sagittal and the coronal diameters of the fontanelle. The broken lines, meeting at *e,* represent the oblique or Elsässer's diameter of the fontanelle.

which there are four, two frontal and two parietal, as an arc of a circle, and the points where the four sutures (the right and left coronal, metopic, and the sagittal sutures) become confluent with the fontanelle as the points at which these arcs become tangent. The figure of the fontanelle under these conditions would be that of a symmetrical area bounded by identical curved margins, as shown in the unshaded segment of figure 4. The relation of the area of this figure to its shortest diameters, which would correspond to the Elsässer's diameters of the fontanelle, may be computed by simple geometry.

As seen in this figure:

$$
\begin{aligned}
2\overline{OC}^2 &= (\overline{OC} - \overline{em})^2 \\
\overline{OC} &= (1+\sqrt{2}\,\overline{em} \\
\square\overset{\frown}{ABCD} &= 4[\square BOCe - \square BOCm] \\
&= 4\left[(1+\sqrt{2})^2\overline{em}^2 - \frac{(1+\sqrt{2})^2\pi\overline{em}^2}{4}\right] \\
&= (3+2\sqrt{2})(4-\pi)\overline{em}^2 \\
&= 5.002\,\overline{em}^2 \\
&= 1.251\,\overline{ab}^2
\end{aligned}
$$

and since ab is equal to the mean oblique diameter (D), A (the area) $= 1.251\ D^2$.

TABLE 6

Observed median fontanelle area and fontanelle area calculated from the expression $A = D^2$

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		DIFFERENCES BETWEEN (a) AND (b)		RATIO OF DIFFERENCE TO S.Q. OF MEDIAN
Interval	Median	Median (a)	Calculated value ($A = D^2$) (b)	*Sq.cm.*	*Per cent*	
0.0 to 0.5	0.400	0.19	0.16	+0.03	+18.75	..
0.5 to 1.0	0.725	0.67	0.52	+0.14	+27.47	4.73
1.0 to 1.5	1.250	2.00	1.56	+0.44	+28.00	8.16
1.5 to 2.0	1.730	3.67	2.99	+0.68	+22.62	10.21
2.0 to 2.5	2.230	6.58	4.97	+1.61	+32.32	15.02
2.5 to 3.5	2.800	9.58	7.84	+1.74	+22.19	4.45

Number and distribution of observations as given in table 2.

The results obtained by applying this geometrical formula to the mean diameters of the material at hand are shown in table 6. The calculated values are, with one exception, below the observed ones; the root mean-square deviation of the calculated from the corresponding observed values is 0.76 sq.cm., the mean weighted absolute deviation is 0.57 sq.cm., and the mean weighted relative deviation is 14.28 per cent. The ratios of the differences between the calculated and corresponding observed values to the probable errors of the latter average

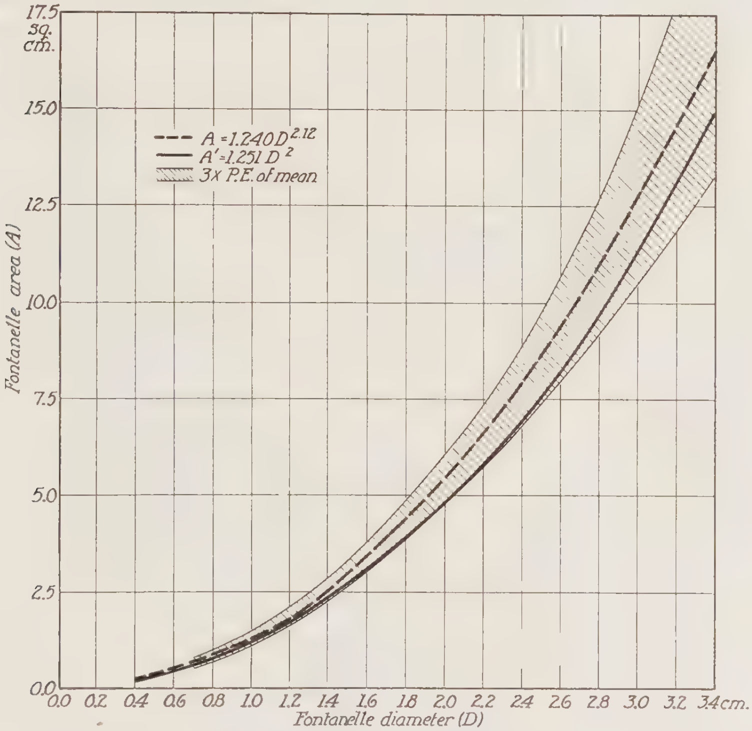

Fig. 5 A graphic comparison of geometric and empirical expressions for the relation between fontanelle area and the mean oblique (Elsässer's) diameter of the fontanelle in infancy. Broken line, empirical expression; solid line, geometric expression; shaded area, three times probable error of observed means (for 0.5-cm. intervals of fontanelle diameter) placed about the values obtained by the empirical expression.

3.01 and range from 1.08 to 4.15. Thus the mean ratio lies at about the commonly accepted margin of significance of a difference. This relationship is shown in graphic form in figure 5.

As pointed out in a preceding paragraph, there is some skewness in the distribution of areas of fontanelles in respect

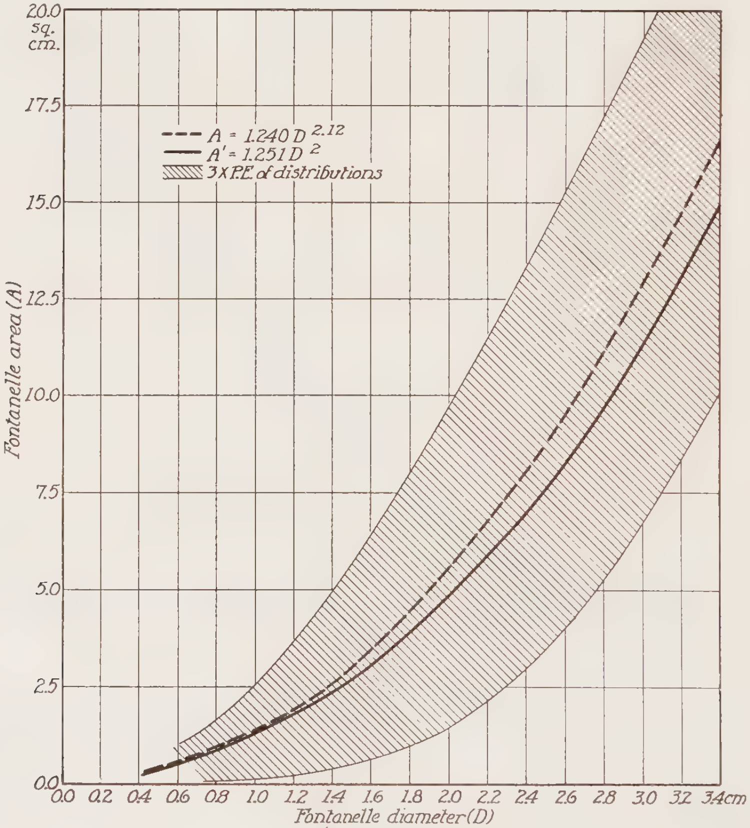

Fig. 6 A graphic comparison of the geometric and empirical expressions for the relation between the fontanelle area and the mean oblique (Elsässer's) diameter of the fontanelle in infancy. Broken line, empirical expression; solid line, geometric expression; shaded area, three times the probable error of distribution of observations, compiled for 0.5-cm. intervals of fontanelle diameter, placed about the values obtained by the empirical expression.

TABLE 7

Observed mean fontanelle area and fontanelle area calculated from the expression $A = 1.251\ D^2$

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		DIFFERENCES BETWEEN (a) AND (b)		RATIO OF DIFFERENCE TO P.E. OF OBSERVED MEAN
Interval	Mean	Observed mean (a)	Calculated value ($A = 1.251\ D^2$) (b)	*Sq.cm.*	*Per cent*	
0.0 to 0.5	0.400	0.190	0.200	−0.010	− 5.00	..
0.5 to 1.0	0.734	0.748	0.674	+0.074	+11.04	2.02
1.0 to 1.5	1.225	2.126	1.877	+0.249	+13.25	3.24
1.5 to 2.0	1.710	4.051	3.658	+0.393	+10.75	4.15
2.0 to 2.5	2.189	6.693	5.994	+0.698	+11.65	3.73
2.5 to 3.0	2.627	10.428	8.633	+1.794	+20.78	3.85
3.0 to 3.5	3.092	12.904	11.960	+0.944	+ 7.89	1.08

Computations of differences and ratios made from values carried one place farther than given in this table. Number and distribution of observations as given in table 1.

TABLE 8

Observed median fontanelle area and fontanelle area calculated from the expression $A = 1.251\ D^2$

FONTANELLE DIAMETER (CM.)		FONTANELLE AREA (SQ.CM.)		DIFFERENCES BETWEEN (a) AND (b)		RATIO OF DIFFERENCE TO S.Q. OF MEDIAN
Interval	Median	Median (a)	Calculated value ($A = 1.251\ D^2$) (b)	*Sq.cm.*	*Per cent*	
0.0 to 0.5	0.400	0.19	0.20	−0.01	−5.00	..
0.5 to 1.0	0.725	0.67	0.66	+0.01	+1.52	0.33
1.0 to 1.5	1.250	2.00	1.95	+0.05	+2.56	0.93
1.5 to 2.0	1.730	3.67	3.74	−0.07	−1.87	1.06
2.0 to 2.5	2.230	6.58	6.22	+0.36	+5.79	3.36
2.5 to 3.5	2.800	9.58	9.81	−0.23	−2.34	0.59

Number and distribution of observations as given in table 2.

TABLE 9

Fontanelle diameter and calculated fontanelle area

FONTANELLE DIAMETER (CM.)	FONTANELLE AREA (SQ.CM.) AS COMPUTED FROM THE EXPRESSIONS:		
	(1) $A = D^2$	(2) $A = 1.251\ D^2$	(3) $A = 1.24\ D^{2.12}$
0.5	0.25	0.31	0.28
1.0	1.00	1.25	1.24
1.5	2.25	2.81	2.93
2.0	4.00	5.00	5.53
2.5	6.25	7.82	8.65
3.0	9.00	11.26	12.73
3.5	12.25	15.32	17.65

to their diameters, and when the effect of this skewness is partially removed from the measure of central tendency by the use of the median in place of the mean, lower values result that fall in a fairly regular sequence. It seemed desirable to test the geometric formula just described by applying it to these medians which may represent a truer measure of a normal measurement of the fontanelles than do the arithmetic means.

This comparison is shown in figure 1 and table 7. Here we find a striking agreement. The root mean deviation of the calculated from the corresponding observed (median) values is 0.19 sq.cm. The weighted mean deviation, taken without regard to sign, is 0.14 sq.cm. and the corresponding relative deviation is 4.94 per cent. The plus and minus deviations are equally distributed as to number. The ratio of the differences between the calculated geometric values and the observed median values range from 0.33 to 3.36 and average 1.25.

It appears, then, that if the weight of observations with large fontanelle areas but without correspondingly increased diameters is reduced by the use of the median rather than the mean, the fit of the theoretic geometric formula is an excellent one.

SUMMARY

1. A series of tracings of fontanelles of 325 infants under one year of age have been measured with respect to their areas and their mean oblique (Elsässer's) diameters. The relationships of the means of the two dimensions may be fairly well represented by the expression, $A = 1.24\ D^{2.12}$.

2. The simple approximation, $A = D^2$, has proved entirely unreliable as an expression of this relation, but the formula $A = 1.251\ D^2$, which is based upon a more elaborate geometric convention, gives a good fit to the observed central tendencies, particularly when these tendencies are measured by the medians rather than the arithmetic means of area for given intervals of fontanelle diameter.

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A SURFACE STRUCTURE (?) IN NORMAL NUCLEATED ERYTHROCYTES

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TWO FIGURES

In studying erythrocytes of the blood of animals in which these cells are found in circulation in the nucleated form, a 'stigma' was observed when the cells were 'supravitally' stained and kept in the wet condition.[1] This structure apparently does not fit into any category of the many 'granules' previously described. In using brilliant cresyl blue as a supravital stain in the study of the blood of the frog, the pigeon, the horned toad (Phrynosoma solare), and the snake, this stigma stained a deep blue and was situated on the surface of the cell, eccentrically. It was a constant finding on each and every erythrocyte, it being the same size or slightly smaller than the specific granules of a human eosinophile. As a rule, it was situated on the upper surface of the cell. Its periphery was sharply outlined, in contrast to the granules observed within the cytoplasm in connection with, or independently of, the reticulum. When the cells were stained under the microscope and the different stages of staining were followed by the observer, this stigma was the first to take the stain; then the nucleus was stained; that was followed by the reticulum and, lastly, by the appearance of another stigma. This second stigma was smaller than the first, and in small, nucleated erythrocytes it could not be distinguished very readily, unless one had been accustomed to its observation in larger cells. In the smaller erythrocytes (pigeon) both stigmata were easily confused with the granules that appear within the meshes of the reticulum, but the larger one, owing to its property of taking up the stain first (or alone, when there is not sufficient quantity to stain any part of the reticulum), could always be distinguished from any other element of the erythrocyte. The second stigma is generally smaller than the first and it lies on the opposite surface of the cell—as a rule, the lower. Its size is about one-half or three-fourths of that of the first stigma. The two stigmata never appeared to be situated on the same plane, and when one was visible, the other was out of focus. The distance between the two, as estimated by the focusing of the microscope, was similar to the approximate thickness of the erythrocyte. They were a constant finding on every nucleated reticulated erythrocyte. They were first observed in wet, supravitally stained preparations that were prepared by the following technic.

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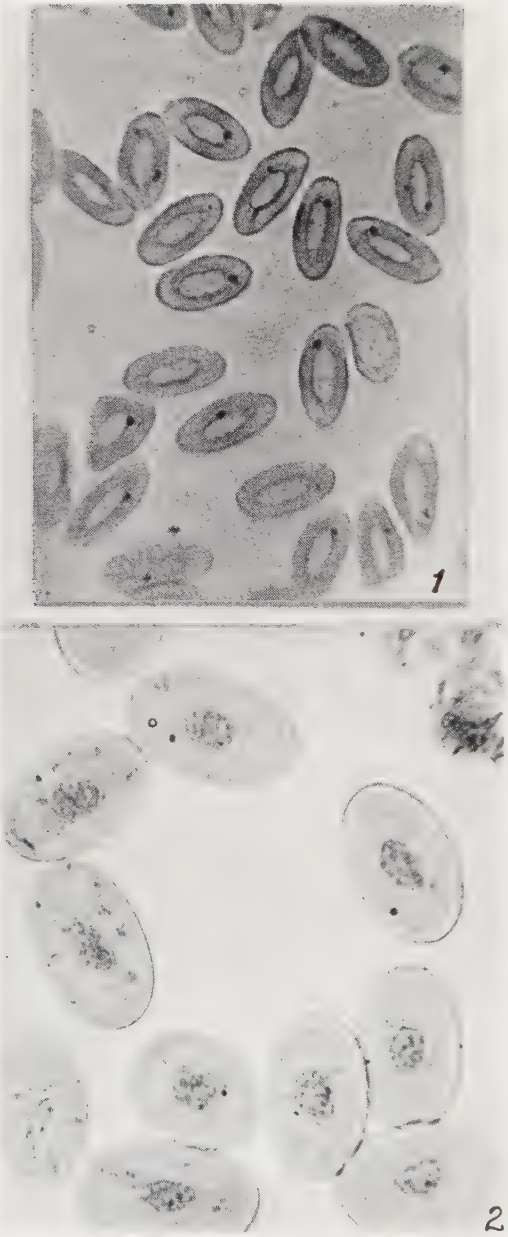
[1] Stigma is the Greek word for 'mark' or 'dot.' In this paper it is used as a general descriptive term and does not imply any particular morphological significance.

METHODS

With a diamond point a groove was made on an ordinary slide, extending from the one end to the middle. This was widened and deepened with the corner of another slide, the end of the groove being made as smooth as possible. A small drop of blood was placed on a cover-slip, which was inverted and dropped on the slide, so that the drop of blood fell on the end of the groove. It was found of great importance to have a drop of blood of minimal size, so that the blood would not spread to the edges of the cover-slip. A drop that ultimately covers but one-third of the cover-slip's surface is best suited for these studies. The end of an ordinary medicine dropper was drawn to a long capillary pipette as thin as could be obtained. A drop of stain was deposited into this pipette. The end was then inserted into the groove on the slide between cover-slip and slide and, by means of the rubber bulb of the dropper, a minute droplet of stain (usually so small that it was barely visible) was squeezed into the mass of the cells. Additional injections of stain were added from time to time, as the cells that lay around the stain were studied.

A second method used was as follows: A small drop of blood is placed on the cover-slip and this inverted and dropped onto an ordinary slide. (As before, the blood should not spread to the edges of the cover-slip.) The specimen is brought under the microscope and under observation. A drop of the brilliant-cresyl-blue solution in 0.85 per cent sodium chloride is deposited on the slide near the edge of the cover-slip. The stain readily spreads under the cover-slip and surrounds the blood. Although the cells at the outer rim take the stain too readily, one can follow the different stages of staining by moving the field from the edge to the middle of the blood-covered area.

The larger or first stigma to appear is best demonstrated in the following manner: To a small number of erythrocytes suspended in their serum, brilliant-cresyl-blue saturated solution in 0.85 per cent sodium chloride is added drop by drop. A few cells are examined microscopically with every additional amount of stain. It will be observed that when the suspension takes a slightly bluish tinge, the cells exhibit a brightly stained blue stigma, while the reticulum and the nucleus are as yet wholly unstained. A good result is obtained when 0.40 cc. of the saturated brilliant-cresyl-blue solution is mixed with 2.0 cc. of blood. The number of erythrocytes present is not important, as the staining capacity of the dye was found to be proportional to the total volume of the blood, and not to the number of cells present. (Figure 1 is a film from such a suspension of pigeon's blood. Compare the brightly stained stigma with the unstained nucleus and reticulum.)



Figures 1 and 2

OBSERVATIONS

With these procedures of staining, it was noticed that when the stain was present in minute quantities, the cells exhibited one brightly stained stigma that, as a rule, lay on the upper surface and favored the smaller axis of the cell. When more of the dye was injected, there appeared a thread-like line of particles that extended from underneath this stigma to the nucleus. After the reticulum appeared, this line or thread could not be separately identified. Occasionally small 'granules' interrupted the course of this thread. These granules were usually slightly smaller and, in contrast to the first stigma, showed lively brownian movements. The nucleus then took the stain. But not until it was completely dyed did the reticulum start taking the stain. This process was followed and apparently completed by the appearance of the other stigma, which as a rule was smaller and lay on the lower surface of the erythrocytic 'membrane.' When the cells were not too heavily stained, the first stigma was much more deeply stained than the rest of the cell (fig. 2). Every single, reticulated, nucleated erythrocyte presented these stigmata and took the stain in this identical manner, irrespective of the species of the animal (frog, pigeon, horned toad, or snake).

In human blood the small number of the nucleated erythrocytes found in circulation made this kind of study, and hence the demonstration of these stigmata, impossible. They could

Fig. 1 Pigeon's blood. Dry film. Two cubic centimeters of pigeon's blood were mixed with 0.40 cc. saturated solution of brilliant cresyl blue in 0.85 per cent sodium chloride. The stigma is deeply stained, while the nucleus has not yet taken the stain.

Fig. 2 Snake's blood. Wet preparation. 'Supravital' staining with brilliant cresyl blue. The red blood cells are completely stained. The nucleus and reticulum are not as heavily stained as the stigma, which appears almost black. Owing to the fact that this was a wet preparation with the cells floating in the fluid, the stigmata of all the cells could not be brought into focus at one time.

The photographs are not retouched and therefore the pictures show some extracellular particles. The stigmata, however, can easily be recognized from their much deeper color.

not be demonstrated in reticulated human erythrocytes, but their existence was suspected. The human reticulated erythrocyte when found in circulation is an abnormal cell, in contrast to the erythrocyte of amphibian, reptilian, and avian origin, where the nucleated and reticulated cell is the adult form. When they are present in sufficient numbers to make the observation practicable, as in pernicious anemia during early remission, the erythrocytes are largely of pathologic shape and this distortion greatly hinders the search for similar structures.

In pigeons, following an intravenous injection of 5.0 cc. of saturated solution of brilliant cresyl blue in 0.85 per cent sodium chloride (the birds died during the injection and the blood was removed from a large blood vessel or directly from the heart), all the red blood cells exhibited one brightly stained stigma and not one showed a stained nucleus or reticulum. When more stain was added to these cells, they stained in the usual way.

In specimens stained supravitally first and then with the Wright's stain, these stigmata are not recognizable. It appears that they lose the stain during the process of the Wright staining.

For further study of these findings, some supravitally stained wet specimens of blood from a horned toad were sealed with petrolatum or Canada balsam and kept in the icebox. Canada balsam was the more practical to use and it did not seem to affect the cells differently than the petrolatum, for the dry borders of the blood seemed to prevent its mixing with the cells that were still in the wet state. On subsequent examinations, it was found that some of the cells were laked, while the reticulum, the stigmata, and the nucleus were preserved in excellent condition. These cells offered an excellent opportunity for study of the relation of these stigmata to the nucleus and the reticulum. It was plainly apparent that the first and brighter and usually upper stigma was connected with the nucleus by means of a thin thread that seemed to be part of the reticulum.

The larger size of the cells of the horned toad and the clearer arrangement of the meshes of their reticular net permitted a better study of these stigmata, and it was in these cells that the observation was first made. However, all the observations upon the erythrocyte of the horned toad were readily confirmed in the amphibian and the avian erythrocyte.

DISCUSSION

Undoubtedly the first stigma described above has been seen by other observers, either alone or together with other 'granules' that were erroneously thought to be of similar structure and nature. It appears that the first observer was Schultze(1), who put live triton larvae in aqueous methylene-blue solutions of from 1:100,000 to 1:1,000,000. He described a blue granule that could also be seen in the unstained blood of the same larvae. Similarly, Fischel(2) experimented with live amphibian larvae by immersing them in weak aqueous solutions of vital stains. In figure 33 of his paper he shows a blood vessel filled with red blood cells each containing a small number of stained dots. One of them must of necessity be identical with the one here described, but it is not separately recognized by the author. It would not be necessary to discuss the nature of the findings described previously by Bremer(3) and Dehler(4) if their observations had not been misinterpreted by other authors. Bremer used unstained preparations of turtle blood, while Dehler made his studies on the blood of the chick embryo in paraffin-fixed tissue. Since the stigmata do not appear in fixed specimens, and the pictures shown by Dehler do not even remotely suggest any similarity or resemblance to stigmata, and inasmuch as Bremer later(5) identified Dehler's findings with his own, it is believed that the structures described by these authors do not correspond to the ones here described. In the author's opinion, Bremer's findings are exactly identical with Ranvier's(6) 'vacuoles spheriques' observed in the fresh blood of the frog. Both Bremer and Dehler agreed that the structures they observed represent a form of centrosome. Such 'centrosomes' Apáthy(7) observed in the blood of full-grown salamanders in air-dried, fixed, and stained specimens. Weidenreich(8) considered the granules or globules observed by himself and others as artefacts. Meves(9), experimenting with the blood of salamanders vitally stained with methylene blue, apparently described the larger stigma correctly, but stated that "instead of one only, one observes frequently two or three and even a greater number of corresponding smaller granules (Kügelchen)." He called the whole group 'chromatoid granules' and identified them with the structures observed by Apáthy, Schultze, and Fischel, but disagreed with them and also with Bremer and Dehler in interpreting them as 'centrosomes.' He thought that they might represent "rejected and 'corrupted' nucleoli." In any case, their strong reaction to the supravital stains was for him a proof of their being 'dead elements.' Even in more recent articles this structure has been confused with many elements from which it could easily be separated. Schilling (10) described 'small, dark chromatin grains' found on his 'Kapselkörper' in man and other mammals and associated them with the granules or groups of granules described by the aforementioned authors. He believed them all to be 'centrosomes.' Jolly(11, 12, 13) noted this vitally demonstrated stigma in normal nucleated erythrocytes as single granules or multiple droplets. He distinguished his structures from the 'brilliant granules' of Ranvier, but identified them with Apáthy's and Bremer's. He suggested that they represented a form of centrosome or that they were formed by transformation of a chromosome. Isaacs'(14) 'refractive granule' as found in non-nucleated erythrocytes is of different nature, whether demonstrated in the fresh state or after the preparation is fixed and stained. Jordan(15), after staining erythrocytes of the frog with neutral red, observed "a variable number of small red-stained spherical granules and globules, some of which exhibited brownian movements." Lewis and Lewis(16), who observed supravitally stained granules in the blood of fish, amphibians, and reptiles, distinguished between 'granules' and 'vacuoles' in their specimens. Chlopin(17) identified the 'few enclosures' that he obtained after placing live axolotls in a weak solution of neutral red over a long period of time with the 'vacuole-like shapes (formations)' that he observed in fixed and stained preparations, as well as with the different structures observed by other authors. Dawson(18, 19) thinks that the pre-existing granules of what he calls ''segregation apparatus of the red blood cells'' are typically grouped as bipolar clusters which may be seen in fresh blood and are readily demonstrated as basophilic bodies in smears stained with Wright's or Giemsa's stains. Although he makes distinction between the pre-existing and the induced granules and globules, he fails to recognize separately the stigmata here described and which cannot be stained or counterstained with the Wright's stain.

The separate identity of the stigma here described to appear first is shown from the following facts: It is single and apparently common to all circulating, mature, nucleated erythrocytes. When more than one was observed, before the nucleus reacted to the stain, the rest were of a different nature as not found uniformly in all cells. It is either on the surface or very near it, as proved by the focusing of the microscope. It never shows brownian movements. Whenever such movements were described, they ought to belong to the granules that lie within the cytoplasm and, according to our observations, within the meshes of the reticulum. Whenever the single stigma was observed moving, the movements were not independent of the whole cell.

In commenting on these findings, it might be more expedient to consider separately the larger stigma. For, unlike the smaller stigma, it cannot be confused with any other element of the erythrocyte. Its property of taking the stain before all other stainable elements and the fact that it can be stained and demonstrated separately and alone prove conclusively and unmistakably its separate identity.

Its affinity for supravital stains would show that it is related to the nucleus or the reticulum. However, unlike those structures, it stains deeply when the stain is present in a solution so weak that it cannot stain the nucleus or the reticular substance. It could be supposed that it reacts to the stain before all other elements because it dies first. Apparently this cannot be the case, for if the specimen is sealed and left over a period of time, it continues to show only these stigmata stained, and no other element. Further evidence pointing in the same direction is the fact that this stigma cannot be demonstrated in specimens counterstained with the Wright's stain.

It could not be proved whether this stigma is on the membrane or immediately below it. If it is on the membrane, it might indicate a hollow cavity where the stain accumulates, or an anomalous surface, possibly the point of separation of the erythrocyte from the mother or sister cell. It is difficult to explain why this particular spot of the membrane reacts to weak solutions of vital stains while the remainder does not.

The observation that this stigma is connected by a thread, probably 'reticular,' with the nucleus and the fact that the nucleus takes up the stain before the reticulum reacts to the stain at all permit one to suppose, from a purely speculative point of view, that a microcanalicular system leads from the surface (membrane) of the cell to the nucleus first, then through the reticular meshes (if the reticulum is a preexisting structure) to the opposite surface of the membrane. This theory could support also the existence of the smaller stigma observed after nucleus and reticulum have been stained. If this be true, one might assume the existence of a minute circulatory system within the erythrocyte, extending from the first and larger stigma (the stoma) to the second and smaller stigma (the anus of the cell): perhaps something of the nature of a Golgi apparatus, but quite dissimilar to the one Schilling(10) suggests. Thus the stain, or the nutritional substances, during life, may enter the cell and be eliminated through a definite route. Then the permeability of the cell 'membrane' would not be a required condition for the explanation of the different physiological phenomena of the erythrocytic cell. Certainly these theories are compatible with the fact that the stained reticulum may be washed free of stain with serum and restained many times, as proved in this laboratory(20).

SUMMARY

A 'structure' in nucleated erythrocytes is here described that stains only supravitally, appearing as a deeply stained stigma that lies on the surface or 'membrane' of the cells. It is found in all the red blood cells of those animals in which nuclei are characteristic of the adult form. It is absolutely distinctive in its identity and is not to be confused with any other 'granules' or 'vacuoles.'

A second stigma is suspected to appear after the staining of the cell is apparently completed. This lies on the opposite side of the cell and is slightly smaller than the first.

The author wishes to express his appreciation to Dr. Cyrus C. Sturgis for supplying facilities and to Dr. Raphael Isaacs for valuable suggestions.

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TOTAL GLOMERULAR COUNTS IN HEMIHYPERTROPHY

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Among others, Arataki(1, 2), Jackson and Shiels(3), and Moore(4) have demonstrated that unilateral nephrectomy during growth does not influence the total number of glomeruli in the kidney of the adult animal. Moore(5) has demonstrated that the enlarged kidney associated with hypoplasia and agenesia contains the usual number of glomeruli for one kidney of that organism. A recent autopsy at the Babies' and Children's Hospital of Cleveland afforded the opportunity to enumerate the total glomeruli in each kidney of a case of so-called hemihypertrophy. Since it is unlikely that another case will be encountered and since the results contribute to the general thesis that the number of glomeruli in the kidney is independent of either acquired or congenital defects in the opposite kidney, it is desirable to report the figures in this one case. Only sufficient clinical and pathological data are given to establish the diagnosis. The details of the methods used are given in a paper by Moore(6). Briefly, it consists of injection of the vascular bed with a mixture of equal parts of 1.5 per cent ferric ammonium citrate and 1.5 per cent potassium ferrocyanide, separation of the cortex and medulla, digestion of the cortex in hydrochloric acid, and enumeration of the glomeruli in weighed aliquot samples.

CASE

Hospital no. 202, autopsy no. 305, a white male, seven years of age, admitted to Babies' and Children's Hospital of Cleveland because of hemorrhage from the rectum. Since eleven months of age, the right side of the body had grown more rapidly than the left. Measurements shortly before death gave the following:

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[1] Hanna Research Fellow in pathology.

Part	*Right Cm.*	*Left Cm.*	*Part*	*Right Cm.*	*Left Cm.*
Elbow circumference,	16.2	14.0	Calf circumference,	18.1	16.7
Wrist circumference,	15.5	10.8	Knee circumference,	28.0	32.0
Arm length,	49.0	45.0	Ankle circumference,	17.5	15.7
Leg length,	60.0	61.5	Arch circumference,	19.5	17.5
Thigh circumference,	26.0	21.8			

During the past two years, a general physical and mental decline has been interrupted by an acute suppurative mastoiditis and a severe prolapse of the rectum. The clinical diagnosis was: hemihypertrophy; prolapse of rectum; secondary anemia; internal hemorrhoids; dental caries; chronic suppurative right mastoiditis; multiple subcutaneous lipomata; and mental retardation.

The anatomical diagnosis was: relative enlargement of the right kidney, right adrenal, right testicle, right lobe of thyroid, right cerebral hemisphere, and right half of the spinal cord; chronic inflammation and varices with ulceration of the sigmoid and rectum; confluent bronchopneumonia of the right lower and right middle lobes; varicose veins of the extremities and walls of the ureter; vascular naevi of hands and feet; hydro-ureters and hydronephrosis; retention cysts of right kidney.

The organ weights of paired organs were as follows:

Organ	*Right Grams*	*Left Grams*	*Per cent right larger than left*
Lung,	430	400	7.5
Adrenal,	3.5	3.0	16.6
Kidney,	195	150	30.0
Testicle,	4	1.5	166.6

Right kidney. Total cortex, 51.6 grams

SAMPLE	WEIGHT, GRAMS	COUNT	GLOMERULI, PER GRAM	PER CENT VARIATION
1	1.47	25,389	17,271	—1.6
2	1.31	23,438	17,892	+1.8
Total	2.78	48,827		

Average glomeruli per gram,	17,563
Estimated total glomeruli in kidney,	906,251

Left kidney. Total cortex, 53.47 grams

1	2.27	34,710	15,291	—3.3
2	1.83	30,169	16,486	+4.1
Total	4.10	64,879		

Average glomeruli per gram, 15,824
Estimated total glomeruli in kidney, 846,109

It is impossible to compare the two cortices, since the weights were taken after injection and preliminary digestion and are not comparable. The total number of glomeruli in the right kidney exceeds the number in the left kidney by about 5 per cent.

It has been demonstrated by Vimtrup(7) in the cat, by Hayman and Starr(8), Moore and Lukianoff(9), and Nelson (10) in the rabbit, and by Moore(6) in man, that the glomeruli in each kidney of any one animal rarely differ more than 10 per cent. Hence, the difference in this case may be regarded as within the normal range and the conclusion drawn that the enlargement of the right side of the body had no effect on the total number of glomeruli in the right kidney.

The capillary loops of numerous glomeruli from both the right and the left kidney have been studied with the aid of a Chambers microdissection apparatus. Glomeruli from both kidneys contained from thirty to forty-five capillary loops with three to five primary branches of the afferent vessel, which is within the normal limits found by Vimtrup(7) and this laboratory.

No data on the sizes of the glomeruli and tubules are available, since the entire kidney parenchyma was used for injection.

SUMMARY

1. In a case of hemihypertrophy, both kidneys contained essentially the same number of glomeruli, in spite of the notable difference in size and weight of the two organs.

2. The internal vascularization of the glomeruli of the enlarged kidney did not differ from that of the opposite kidney or normal kidneys.

I wish to thank Dr. C. T. J. Dodge for the clinical record and Dr. H. T. Karsner for aid in the preparation of the manuscript.

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BILATERAL OVARIES IN COOPER'S HAWK, WITH NOTES ON KIDNEY STRUCTURE

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ONE FIGURE

OVARIES

The structure of the ovary or the ovaries of the bird is a proposition that occasionally attracts attention in the study of comparative anatomy. It appears that both the right and left ovaries begin to develop in the embryo, but that only the left persists in many species. However, in a limited number of species, bilateral development of the adult ovaries is known to exist, although the right ovary is generally somewhat reduced in size.

Thomson[1] sums up the situation as follows:

> Descending from theory to fact, we should notice that the unilateral development is not absolute. Max Kollman reports (1919) that in six cases among falcons (four of *Accipiter nisus* (sparrow hawk) one of *Astur palumbarius* (gos-hawk) and one of *Tinnunculus tinnunculus* (kestrel), paired ovaries were found. Yet in these cases the right oviduct remained quite vestigial, which is a little puzzling. In a duck observed by Chappellier there were two ovaries *and* two oviducts, and the bird sometimes laid two eggs in a day.

Snyder[2] reports double ovaries in the marsh hawk (Circus hudsonicus) and states that they were "lying ventral to the kidneys and distinctly separated from each other on the two sides of the median vertical plane." He further states that "The follicles of both ovaries were enlarged giving the appearance of a functional condition in both organs''; also, that the specimen ''has recently been examined by Dr. Alexander Wetmore who remarks that this condition is known to be of regular occurrence in some forms of Hawks, particularly in the female Sharp-shinned Hawk among American species.''

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[1] Thomson, J. A. 1923. The biology of birds, p. 245.

[2] Snyder, L. L. 1928. Double ovaries in Circus hudsonicus. Auk, N.S., vol. 45, p. 98.

The writer recently examined two specimens of Cooper's hawk (Accipiter cooperi), both of which had two ovaries and but one oviduct, the left. The accompanying figure illustrates the size and position of the ovaries. It will be observed that they were distinctly separated in the midline; in fact, a blood vessel and some connective tissue filled the small space between them. In both cases the left ovary was slightly more anterior in position and slightly larger than the right ovary. There were large follicles in both ovaries, but they were more numerous in the left ovary. However, the smallest follicles in the right ovary appeared to be larger than the smallest follicles in the left ovary.

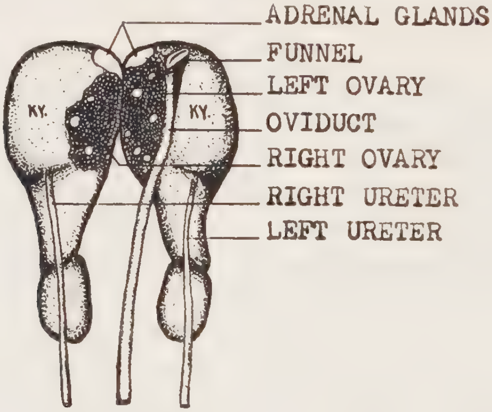

Fig. 1 The urinogenital organs of Cooper's hawk. × 1.

The oviduct (left) extended from the middle compartment of the cloaca (urodaeum) and along the outer margin of the left ovary to the anterior end of the kidney, where its funnel opened on the ventral surface. It was not convoluted, but, on the contrary, fairly direct in its course. There may have been a vestige of a right oviduct, but such was not observed in either specimen.

KIDNEYS

Text-book descriptions of bird kidneys usually refer to those of the chicken or the pigeon. They are illustrated as three-lobed organs lying more or less within the hollows of the pelvis. The lobes are generally figured as being about equal in size.

It will be observed that in Cooper's hawk the lobes of the kidneys are distinctly unequal in size, the anterior lobe being larger than the combined mass of the other two lobes in each case. Also, the posterior lobe of each kidney is practically separated from the middle lobe, being connected to the latter by a ureter and a blood vessel. The general shape of the kidneys is indicated in the accompanying illustration.

The ureters appear on the ventral surfaces of the middle lobes of the kidneys. They remain in contact with the ventral surfaces of the posterior lobes and then proceed posteriorly in contact with the dorsal body wall to separate openings into the urodaeum. The latter are slightly posterior to the opening of the oviduct.

ROW FORMATION AND OTHER TYPES OF ARRANGEMENT OF CARTILAGE CELLS IN ENDOCHONDRAL OSSIFICATION

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SEVEN TEXT FIGURES AND TWO PLATES (EIGHT FIGURES)

It is a well-known fact that in the process of endochondral ossification the cells of the cartilage usually become arranged in rather definite rows parallel to the long axis of the bone, and that they become greatly enlarged just in advance of the marrow which removes the cartilage and deposits bone matrix. I have not, however, been able to find a definite statement of the method by which these rows are formed, either in text-books or in special papers dealing with ossification.

The statements on the subject are always indefinite and sometimes misleading, it being commonly said that the cells 'become arranged in rows' or that they 'become rearranged.' Apparently, not much attention has been given to this phase of ossification.

The importance of the behavior of the cartilage cells is generally recognized, and has recently been well stated by Stump, as follows:

> The growth impulse affecting the shape, size, and form of the bone is inherent in the cartilage cells, both in diaphysis and epiphysis. Bone formation is a secondary process of replacement and consolidation following in the wake of the cartilaginous growth.[1]

In view of the importance attached to the growth of the cartilage model which precedes the bone and determines its size and shape, it is surprising that more study has not been given to this phase of the process of bone formation.

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[1] Stump, G. W. 1924. The histogenesis of bone. Jour. Anat., vol. 59, p. 136.

The following studies have been made primarily upon developing bones of dog fetuses and young puppies, but comparisons have constantly been made with material from cat, pig, and man. The formation of cartilage-cell rows has been studied and some attention also given to other forms of aggregation of cartilage cells in endochondral ossification.

The formation of rows is seen at its best development in the shafts of rapidly growing bones, such as those of the legs and feet, during the time when they are growing in length by the steady advance of ossification toward the cartilaginous ends of the bones, both before and after the appearance of the epiphyseal centers of ossification.

PRIMITIVE CARTILAGE

In the cartilaginous ends (the epiphyseal regions) of young bones, before the appearance of the epiphyseal centers, the condition of the cartilage is that of a primitive type of hyaline cartilage, such as would be found elsewhere in the body, with no relation to the process of ossification (figs. 8 and 15). The cells are small and all very much of the same size. They have a rounded form. They are undergoing mitotic divisions which take place in all planes. Following the divisions, the pairs of daughter cells slowly migrate apart and soon come to occupy separate lacunae. For this reason the cells of this region occur singly, except for those, here and there, which, on account of recent division, occur in pairs. The growth of the cartilage in this region is not rapid, nor is it conspicuously greater in any one direction. It is simply sufficient in amount and of a suitable nature to keep the cartilaginous ends of the bones of proportionate size and of proper shape. This is the primitive type of cartilage-cell arrangement from which the rows are derived. It might also be remarked, in passing, that in this region the cartilage also receives increments from the mesenchyme which underlies the perichondrium, as well as from the interstitial growth just described.

LONGITUDINAL ROWS

Between the region just described and the advancing ossification front of the diaphysis lies the zone where the cartilage cells are arranged in rows. Figure 15 gives the general setting of the region in a very early stage, while figures 9, 11, and 12 show its structure in greater fullness. This is the zone where the great growth in length of the bone takes place by the multiplication of the cartilage cells in the rows. It is this zone that persists as the epiphyseal cartilage until the termination of the period of growth. Here can readily be observed the process by which the rows of cells become established, as follows:

In this zone, at a definite distance ahead of the advancing marrow, the divisions of the cartilage cells begin to take place in a different manner from that just described for the primitive cartilage of the epiphyseal region. This difference involves four main features: 1) there is a definite orientation of the mitotic figures; 2) the two cells resulting from each division remain close together; 3) instead of retaining the primitive, rounded form, the two daughter cells become greatly flattened, assuming a discoid form; 4) all of these pairs of flattened cells remain oriented in the same way, that is, with their diameters perpendicular to the long axis of the bone. These pairs of flattened cells are the beginning of rows (figs. 9 and 11).

Each of these pairs of flattened cells is derived from a cell which may be termed a 'row-mother cell,' and each pair, by repeated divisions, gives rise to one row of cells. During these divisions, the cells retain the discoid form and maintain the same orientation. Thus the rows are built by the division of flat cells. During the formation of rows, there is no rearrangement of cells, the flattened cells, as they divide, merely retaining a relation like coins in piles. During the process, there are seen intermingled in sections rows of different lengths, some containing two cells, others four cells, eight cells, etc., or numbers intermediate between these if all the cells in the row have not completed the same number of divisions from the row-mother cell (figs. 9 and 11).

It appears that in the formation of rows the divisions of all the cells in a row occur in rather rapid succession, followed by an interval of rest from division. In general, the divisions begin at the end of the row toward the advancing marrow and progress toward the epiphyseal end, but this linear succession of divisions is not strictly maintained.

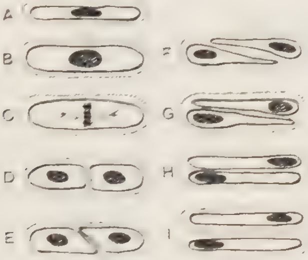
The full number of cells in a row is rather constant for any one ossification front, but different fronts have different characteristic numbers, as shown by the two bones pictured in figures 9 and 11. The numbers of cells in fully developed rows approach very closely to the powers of 2 rather than to some intermediate numbers, such numbers as 4, 8, 16, 32, and 64 being common, the number being determined by the number of divisions which have occurred from the row-mother cell.

By these successive divisions of the cartilage cells in the rows, not only is the number of cells increased, but the actual length of the rows is also increased, and, inasmuch as all the rows have the same orientation, these divisions are an important factor in the elongation of the bone, rather than in its increase in thickness. The increase in thickness comes by multiplication of the number of rows as determined by more numerous row-mother cells produced by the epiphyseal cartilage as the bone elongates. By this method the area of the diaphyseal ossification front grows steadily, keeping pace with the thickness of the shaft as a whole, as it becomes built up by subperiosteal ossification.

Following each division of the cells in the rows, there is formed a thin septum of the cartilage matrix between the two daughter cells, but these transverse partitions remain much thinner than the longitudinal septa which intervene between adjacent rows (figs. 9, 11, and 12).

The flattened form of the cartilage cells is characteristic of the period during which the rows are in process of formation, but when the full number of cells in a row has been produced, then begins the enlargement of the cells, the enlargement in general affecting the cells in sequence, beginning at the diaphyseal ends of the rows (figs. 9, 11, and 12).

In this growth of the cells they do not grow equally in all directions, the growth in thickness being much greater than in width. The result is that the thin, discoid cells become transformed into short cylinders or prisms with length and diameter about equal. During the growth of the cells, they do not undergo any rearrangement, the rows being definitely maintained. The result is that the length of the rows is very greatly increased. Thus, the elongation of the cartilage, which was begun by the division of the flat cells in the rows, is continued with increased speed by the rapid enlargement of these same cells. A study of figure 9 will show many of the features just described.



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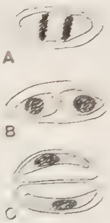

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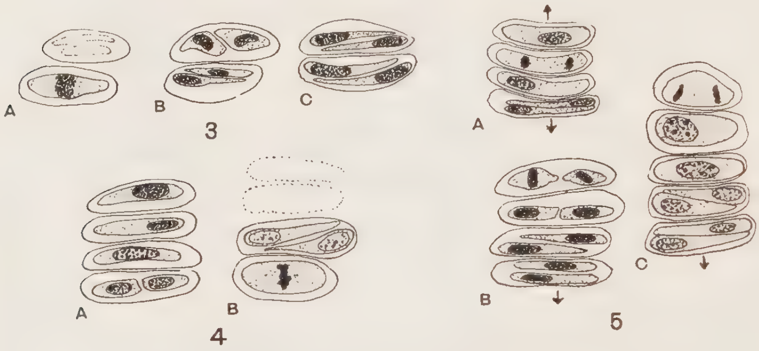
Fig. 1 Diagrammatic drawings to show the mode of division of the discoid cells in rows of cartilage cells in endochondral ossification.

Fig. 2 Camera-lucida drawings of cartilage cells from metatarsal bone of newborn puppy, showing the beginning of row of cells by division of row-mother cell. Outline of lacuna is shown surrounding cells. A. Telophase. B. Two daughter cells just after division. C. The two daughter cells after flattening and assumption of final orientation.

The mode by which divisions of the discoid cells in the rows takes place is as follows: The divisions do not take place in the direction which, at first thought, might be expected. They do not divide directly into two thinner, discoid cells, but into two semicircular cells which lie side by side in the lacuna. That is, the axes of the mitotic figures lie perpendicular to the long axis of the bone. The various stages of these divisions are shown somewhat diagrammatically in figure 1. Following each division, each of the two semicircular cells of a pair soon becomes discoid again by growth of its cytoplasm past its fellow, going through various wedge-shaped stages until the discoid form is attained.

The forms assumed by these cells during these divisions, in rows of various lengths, as actually seen in sections, are shown by camera-lucida drawings in figures 3, 4, 5, and 6. Figure 14 shows a photomicrograph of the discoid cells as seen in cross-section of the rows, including a telophase similar to that shown in figure 6, B, and a pair of semicircular cells like those of figures 4, A, and 6, C.

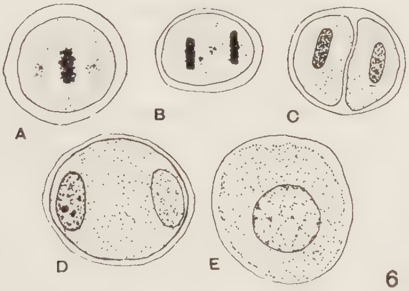


Figs. 3 to 5 Camera-lucida drawings of cartilage cells from metatarsal bones of newborn puppy, showing various phases of cell division, as seen in longitudinal sections of rows. Figure 3, cells from row during multiplication of cells from two to four. Figure 4, cells from row during multiplication of cells from four to eight. Figure 5, cells from row during multiplication of cells from sixteen to thirty-two. Only part of each row shown.

Figure 2 shows the formation of two flattened cells by the division of a row-mother cell, the division taking place in a direction transverse to the long axis of the bone. Thus are produced two cells of rounded form, which by overgrowth and flattening become a typical pair. In some other cases the divisions of the row-mother cells seem to take place in other planes, but if they do, the resulting pairs of cells promptly change their orientation, so that all the flattened pairs become oriented in the same way.

It is also commonly observed that in the two-celled stage of rows the two cells are not strictly discoid, but have rather a concavoconvex form, with the concavities toward each other, as shown in figure 2, C. In the longer rows the form is truly discoid, except that the cells at the ends of the rows are commonly somewhat thickened or concavoconvex.

During the growth of the cells in the rows, their moderate lateral expansion causes the trabeculae between the rows of



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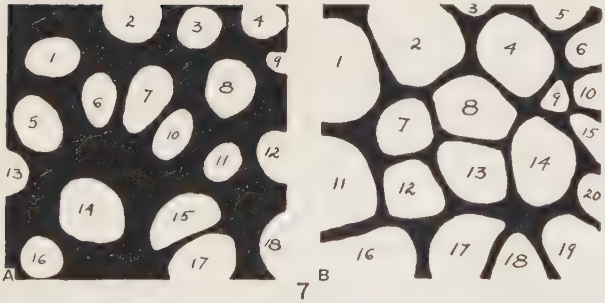

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Fig. 6 Camera-lucida drawings of cartilage cells from radius of young puppy, as seen in cross-sections of the bone. Shows flat surface of discoid cells. A. Metaphase. B. Telophase. C. Daughter cells while still in semicircular form. D. Appearance when two daughter cells have resumed discoid form, the nucleus of the lower cell being seen through cytoplasm of uppermost cell. E. One of the enlarged cells in the diaphyseal end of the rows.

Fig. 7 Camera-lucida outlines from sections cut across the rows of cartilage cells in radius of young puppy, showing enlargement of lacunae and corresponding thinning of matrix between lacunae. A. Section through region of young rows where cells are still flat. B. Section from region of enlarged cells in older ends of rows.

cells to become somewhat thinner than they are where the cells are flattened, though they still remain much more robust than the thin transverse walls between cells within the rows. This thinning of the longitudinal trabeculae is shown in figures 9, 11, and 12. A more definite picture of the extent of the thinning is shown in figure 7, a camera-lucida outline which shows the enlargement of the lacunae and consequent thinning of the intervening matrix, as seen in cross-sections through the rows at two different levels.

It will be recognized that during the rapid growth of the cells in the rows and the consequent increase in the total length of the rows, there must be a corresponding increase in the length of the longitudinal trabeculae between the rows. In this the actual elongation of the growing bone takes place. The question might be raised whether this elongation is accomplished by addition of material to the matrix of the cartilage, or whether the thinning of the trabeculae may be sufficient to compensate for the increase in length, so that the total amount of material in the trabeculae remains the same, and the elongation may be accomplished merely by rearrangement of material already present. Possibly the latter view seems more reasonable, in view of the rapidity of the process and the degenerate nature of the cartilage cells, especially toward the close of the enlargement period.

As far as my observations go—and on this point they are not extensive—when calcification of the cartilage matrix takes place, it affects only the longitudinal trabeculae, the transverse walls between the cells being unaffected by it. These thin, uncalcified walls are seemingly easily destroyed by the invading marrow, as are also the degenerate cartilage cells within the lacunae, so that long, parallel, cylindrical holes are easily produced, as a lining of which the first bone matrix is deposited.

Though the arrangement in rows is perhaps to be considered the characteristic and dominant type of cell arrangement in the cartilage in preparation for the ossification process, there are other types of decided interest. Possibly it may be said that the arrangement in rows characterizes the more rapidly growing regions, while other types are found where growth is less rapid. Row formation may well be considered the highest type of specialization in the process.

CELL NESTS

Another type of arrangement is pictured in figure 13, which represents a longitudinal section through the distal end of a phalanx of the finger of an infant. Here the cells do not pass through the flattened stage and no rows are formed. Instead, the cells are arranged in cell nests, separated from neighboring nests by thick masses of matrix, while the cells within nests are separated by but thin walls. In the proximal end of the same bone the cells are in typical rows, the cells of which pass through the flattened stage (fig. 12). This is the end of the bone where the epiphyseal center will later appear, the end where the greatest growth takes place, and we may well suppose that the difference in cell arrangement of the two ends is definitely correlated with the difference in rate of growth.

The cell nests of the distal end are produced by specialization of primitive cartilage, as are the rows, but the growth is not so predominantly in one direction as when rows are formed. Doubtless each of these groups of cells is derived from a single group-mother cell, just as is each row, the essential difference being that the flattening of the cells does not take place and the cell divisions do not take on a definite orientation.

As the advancing marrow approaches, the cells in these groups enlarge greatly, just as do those of rows, but this enlargement does not result in so pronounced a longitudinal growth of the cartilage as does growth of cells in rows. In such a center there are not formed the straight, longitudinal cylindrical holes, but the invading marrow must follow a somewhat devious course. The resulting trabeculae of primary bone have a correspondingly irregular arrangement, in contrast to the rather straight trabeculae formed when the cartilage cells are in long rows.

A very similar arrangement of cartilage cells is found in epiphyseal centers. Figure 10 shows the epiphyseal center from the same bone as that from which are taken the diaphyseal rows shown in figure 9. This figure shows that there is considerable growth of the cells in the nests prior to the invasion of the lacunae by the marrow, but that the growth of these groups is no greater in one direction than in others. Near the top of figure 10 is the band of primitive cartilage from one side of which develop these nests of cells in the epiphyseal center and from the other side, the rows of the diaphyseal center, the beginnings of which are just included in the picture. This band of primitive cells furnishes the mother cells for both the nests and the rows.

UNIFORM DISTRIBUTION

A still different and more primitive type of cell arrangement occurs at the first formation of the primary center of ossification in each bone. In such a young cartilage model, about midway between the two ends, at a time before either endochondral or subperichondral ossification has begun, the cartilage cells and their enclosing lacunae begin to enlarge. The enlargement continues until, at about the time of the beginning of subperichondral ossification, the lacunae have become so large that they are separated only by thin trabeculae which form an irregular network. These trabeculae are all of about the same thickness; that is, the cells are not arranged in groups of any sort, but are uniformly close together. This is the condition of the cartilage when it is invaded by the first marrow buds from the mesenchyme surrounding the cartilage.

This type of cell arrangement does not persist long in the development of the bone, but soon gives place to the row type (exceptionally to cell nests). A beginning of row formation is present in young cartilage models, even before the deposition of any bone, as seen in figure 15. In such a cartilage, though the central portion, where the first ossification will take place, is of the non-grouped type, toward either end rows have already begun to form, and still beyond this zone is the primitive cartilage of the epiphyseal regions. This primitive cartilage, as long as the bone continues to grow, will furnish mother cells for the development of rows and for the nests of the epiphyseal centers when they appear.

PLATE 1

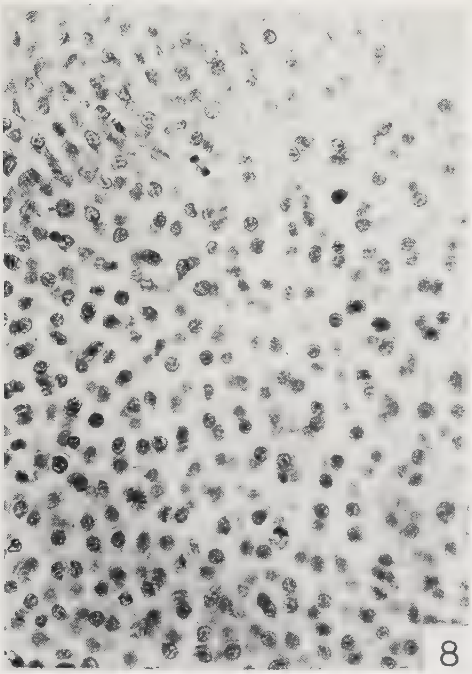
EXPLANATION OF FIGURES

8 Photomicrograph, × 420. From foot of cat fetus, showing primitive form of cartilage in epiphyseal region of cartilage model. The cells are small and have no special grouping or orientation. Two mitotic figures are shown.

9 Photomicrograph, × 187. From longitudinal section through diaphyseal ossification front of radius of young puppy, showing well-developed rows of cartilage cells. Rows with two cells, four cells, and larger numbers are shown, as well as the great enlargement of the cells near the invading marrow.

10 Photomicrograph, × 187. Epiphyseal center corresponding to figure 9. Cells do not pass through flattened stage and are in cell nests instead of rows. Ends of rows of diaphyseal center are shown at top.

11 Photomicrograph, × 187. From metatarsal bone of newborn puppy, showing rows of cells which are shorter than those of figure 9. Pairs of cells which mark beginning of rows are clearly seen.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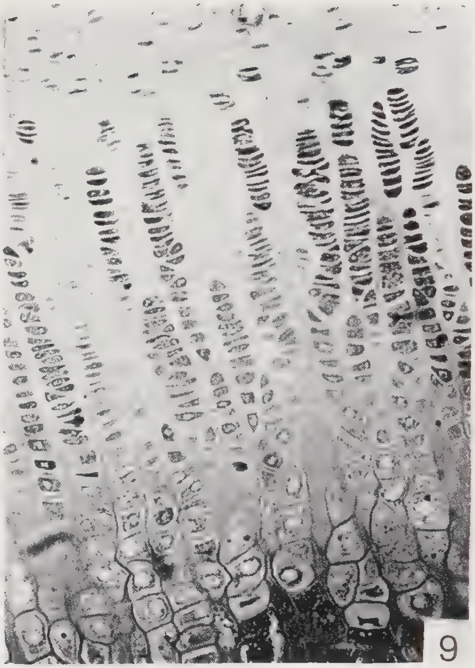
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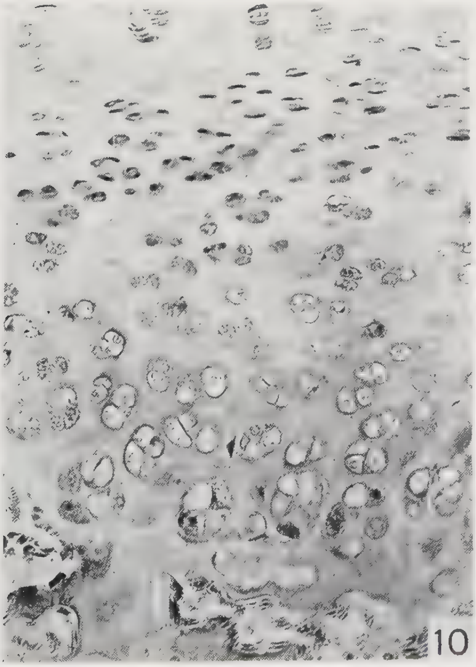
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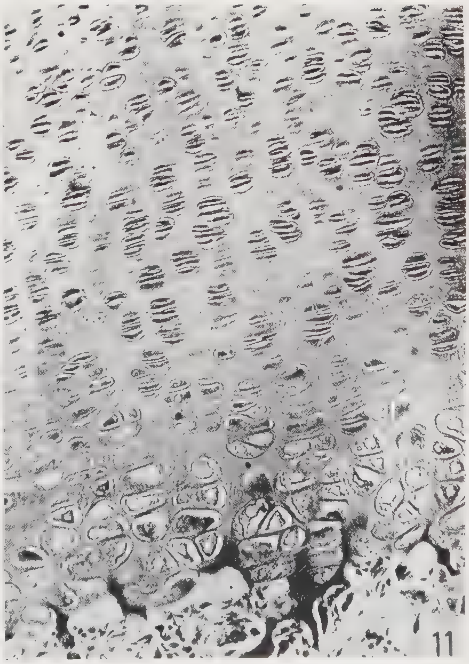
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PLATE 2

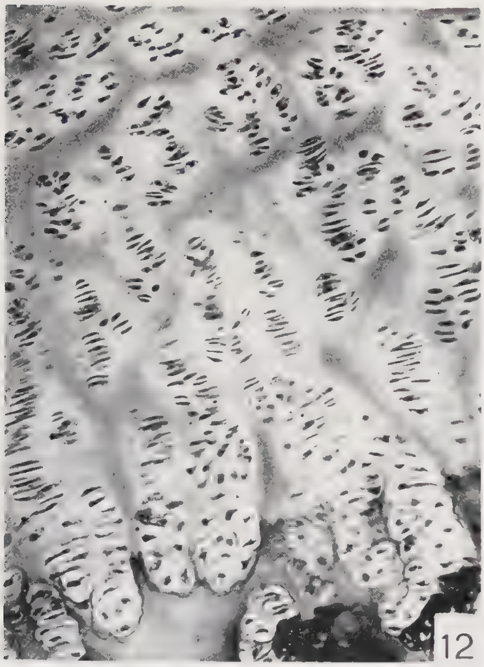
EXPLANATION OF FIGURES

12 Photomicrograph, × 187. From proximal end of phalanx of finger of human infant, showing well-developed rows of cells. This is the rapidly growing end where the epiphyseal center will later appear.

13 Photomicrograph, × 187. From distal end of phalanx of finger of human infant, showing cartilage cells arranged in nests instead of rows.

14 Photomicrograph, × 420. From cross-section of radius of young puppy, showing broad surfaces of discoid cells. Near lower left corner is a telophase, and near the lower right corner, a pair of daughter cells which are still in the semicircular form.

15 Photomicrograph, × 93. From basal phalanx of finger of three-month human fetus, showing specialization of cartilage at the time ossification is just about to begin. Shows enlarged cells at center of cartilage (lower end of picture); short rows just beyond this; and the primitive cartilage of the epiphyseal ends with small, rounded cells.



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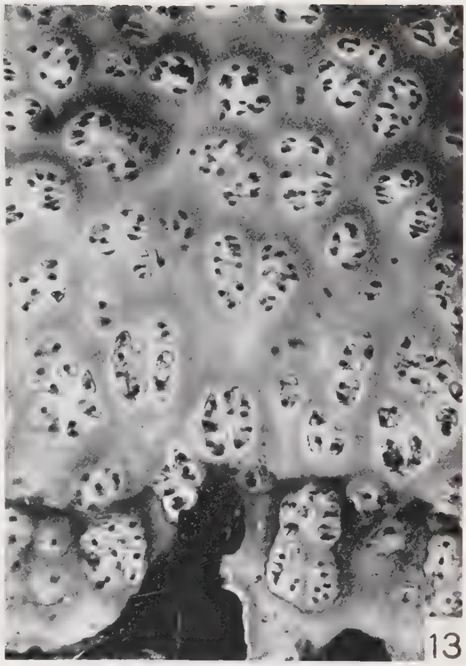

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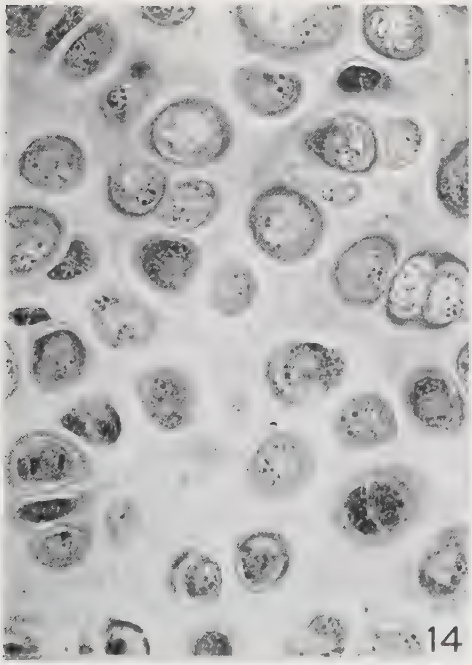

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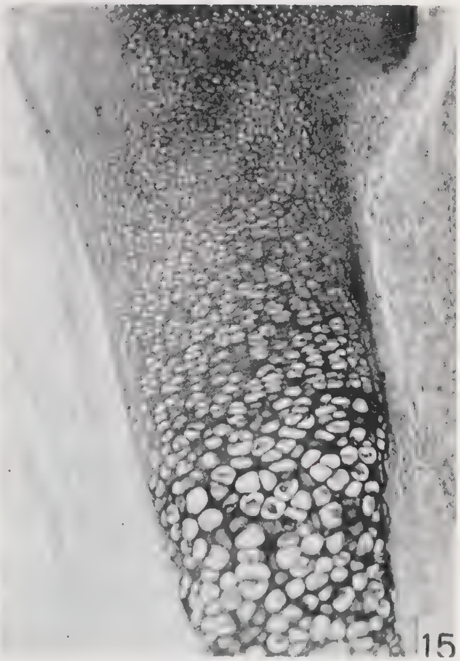

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A QUANTITATIVE STUDY OF THE CAPILLARY SUPPLY IN CERTAIN MAMMALIAN SKELETAL MUSCLES

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PREVIOUS WORK

In 1919, Krogh did the first work on the quantitative study of capillaries in skeletal muscle. With an ocular micrometer, he studied cross-sections of muscle injected with India-ink-gelatin and made capillary counts for two muscles of the frog, two of the guinea-pig, one of the cod, one of the dog, and one of the horse. In 1925, Stoel made a similar study for one red and one white muscle of the rabbit. On the basis of results from two muscles, he suggested a ratio of 2:1 for abundance of capillary supply in the red and white varieties of muscle. Further work was done on rabbit muscle by Duyff and Bouman in 1927. They reported counts on fourteen muscles, but their figures do not substantiate Stoel's suggested ratio.

METHOD

The present work was done on homologous muscles of the rat, guinea-pig, and cat. Capillary counts per unit cross-sectional area of muscle were made. In addition, the following values were computed for each muscle: first, the proportion of muscle tissue to connective tissue in the muscle fasciculi; secondly, the average cross-sectional area of muscle tissue which is metabolically dependent on a single capillary; and thirdly, the average area of transverse sections of muscle fibers.

Capillary counts were made on injected muscle. Animals were etherized, and by cannulation of the iliac artery and ligation of collateral vessels a direct channel to one of the legs was obtained. Following perfusion with a liberal amount of physiological salt solution, injection mass was introduced at a pressure of a 250-cm. column of water. During perfusion and injection the temperature of animal and liquids was maintained between 40 and 45°C. The cut inferior vena cava was used as a drain, and this vessel was clamped intermittently in order to open up additional capillary beds to the injection mass. After injection, blocks of muscle were fixed in 10 per cent formalin, embedded in paraffin, cut in transverse sections, stained with orange G, mounted, and studied. Many animals were injected before the technique was perfected to the point where the results were deemed satisfactory.

Under low magnifying power of the microscope, representative areas of material thus prepared were chosen, and in these areas under high power the individual muscle fibers and capillaries were outlined. A camera lucida was used in all cases and the capillaries and muscle fibers were drawn on 2-mm. cross-section paper at a linear magnification of 745. Capillary counts were made with the aid of a glass plate ruled with a diamond point to give fields of known dimensions at a linear magnification of 745. By placing this plate over a drawing, the capillaries in a rectangular field of known dimensions were counted. In this way a total of 275 different magnified areas was studied. The actual size of these areas varied from 0.005 to 0.04 sq.mm.

The proportion of muscle tissue to connective tissue in transversely cut muscle fasciculi was computed by the following method. The areas representing muscle fibers were cut out from the drawings of the fields in which capillary counts were made. These cut-out pieces of paper were then weighed. This weight, compared with the weight of a piece of the same paper of known area, was used to calculate the actual area of the muscle tissue in a given cross-section area of muscle fasciculi, composed of muscle tissue plus connective tissue. The computed area for muscle tissue was then subtracted from the total area of muscle fasciculi and thus the area for connective tissue was derived, i.e., area of muscle fasciculi minus area of muscle tissue equals area of connective tissue.

The average area of muscle tissue in cross-section which is metabolically dependent on a single capillary was computed by dividing the area of the muscle tissue in a known area of muscle fasciculi by the capillary count of the same area.

The area of transversely cut muscle fibers was arrived at by counting the square millimeters enclosed by the drawn outlines of 600 different individual fibers. For each of the eight muscles an average of seventy-five measurements was computed. By using both methods on several identical areas, it was found that no serious error was introduced by the use of this direct measuring method interchangeably with the indirect weighing procedure described above.

OBSERVATIONS

In the following table are listed the results derived by the methods described above.

ANIMAL	MUSCLE	PER SQUARE MILLIMETER OF MUSCLE CROSS-SECTION			MUSCLE TISSUE IN SQUARE MILLIMETERS DEPENDENT ON ONE CAPILLARY	AVERAGE CROSS-SECTION AREA OF MUSCLE FIBERS IN SQUARE MILLIMETERS
		Capillaries	Muscle-tissue fraction	Connective-tissue fraction		
Rat	Rectus femoris	3573	0.706	0.294	0.000197	0.000609
	Tibialis anterior	3402	0.713	0.287	0.000209	0.000392
Guinea-pig	Rectus femoris	3060	0.707	0.293	0.000227	0.000916
	Tibialis anterior	3501	0.731	0.269	0.000208	0.000752
	Gastrocnemius	2614	0.640	0.360	0.000244	0.000700
Cat	Rectus femoris	2474	0.652	0.348	0.000263	0.000476
	Tibialis anterior	2214	0.686	0.314	0.000309	0.000570
	Gastrocnemius	2341	0.709	0.291	0.000303	0.000580

DISCUSSION

The figures have comparative value only. The amount of edema, which invariably results from gravity perfusion, was kept constant within reasonable limits by subjecting each vascular bed to the same degree of pressure during perfusion and injection. In addition, shrinkage was kept approximately constant by cutting blocks of muscle of equal size from the different animals and by leaving them in the various solutions (fixative, alcohols, etc.) for equal lengths of time.

The fields in which capillary counts were made were small (0.005 to 0.04 sq.mm.). However, by comparison with the average area of the muscle fibers which were contained in these fields—and between which the capillaries were regularly arranged—the matter of size of the field appears in a different light. Thus in the case of the m. tibialis anterior of the rat the average area in which capillaries were counted was 0.0108 sq.mm., while the average cross-sectional area of its muscle fibers is 0.000392 sq.mm. In addition, it should be noted that in each case a representative number of different areas was counted and averaged.

Reference to the table shows that, in general, the amount of capillary supply per unit area of cross-section of homologous muscles is greatest in the rat, intermediate in the guinea-pig, and least in the cat.

The results for the amount of muscle tissue in a unit area of muscle reveal the fact that in each of the eight muscles examined the proportion of muscle tissue in fasciculi approximates 70 per cent. The remaining, approximately 30 per cent, consists of connective tissue.

The discussion of the computations for the area of muscle tissue dependent on a single capillary involves a consideration of metabolic activity. According to Rubner's law, which states that "the metabolism is proportional to the superficial area of an animal" (Lusk, '28, p. 123), the rat has a higher rate of metabolism than the guinea-pig and the latter a higher rate of metabolism than the cat. The figures for the average area of muscle tissue which is metabolically dependent on one capillary show the interrelation of capillary supply to metabolic activity as predicated in Rubner's law. According to this law, metabolism is the most active in the rat, and in this same animal one capillary acts as the nutrient vessel for the smallest area of muscle. In the cat, on the other hand, the animal with predicated least metabolic activity, the area of muscle dependent on one capillary is largest. Intermediate between these two animals is the guinea-pig, both as to metabolic activity and as to muscle area cared for by one capillary.

These calculations indicate that in the muscles of an animal with predicated greater metabolic activity the exchange of nutriment for waste between blood and muscle tissue is more rapid by virtue of the fact that the muscle tissue dependent on one capillary is smaller in amount. Thus an inverse proportion is established between the intensity of metabolism and the area of active tissue supplied by a single capillary.

The figures in the last column of the table are values for the average cross-sectional area of muscle fibers. Each value was computed from seventy-five measurements. In taking these measurements the work of Huber on rabbits and roosters was not overlooked. Huber ('16, pp. 149–168) showed that skeletal muscle fibers have blunt ends at tendinous attachments, but have filamentous intrafascicular terminations. It follows from his observations that if a muscle fiber has no tendinous attachments it is a spindle-shaped structure.

For the reasons indicated, fibers of all sizes in cross-section were chosen at different levels of each muscle. In doing this, great variations in cross-sectional area were observed in different fibers of the same muscle; e.g., in the m. rectus femoris of the rat the largest observed cross-section area of a fiber bore a ratio to the smallest of 343 : 8.

The average figures show that in each animal distinct variations in average area of muscle fibers occur. The muscle fibers in the guinea-pig average appreciably larger in cross-section than either those of the rat or the cat.

CONCLUSIONS

1. In general, the muscles of the rat are more richly supplied with capillaries per unit of cross-section area than are those of the guinea-pig, and these, in turn, than those of the cat.

2. In the muscles studied in these three different animals the number of capillaries per square millimeter of cross-section of muscle, including the connective tissue, varies from 2341 to 3574.

3. In different skeletal muscles of the same animal and in homologous skeletal muscles of different animals the ratio of muscle tissue to connective tissue is approximately constant in a cross-section, whatever the size of muscle fibers. In tissue prepared by the paraffin technique this ratio is approximately 7 : 3.

4. An inverse proportion exists between the predicated intensity of metabolism and the area of muscle tissue supplied by one capillary.

5. Muscle fibers in the guinea-pig are, in general, considerably larger in cross-section than those of the rat and cat. The skeletal muscle fibers in the rat and cat are, on the average, approximately equal in cross-section area.

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